Sexual size dimorphism in damselflies, dragonflies and birds: function and development

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Sexual size dimorphism in damselflies, dragonflies and birds: function and development

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Summary

Differences in size between the sexes are ubiquitous throughout plant and animal taxa. These differences in size (termed sexual size dimorphism, SSD) are often the most conspicuous differences between males and females, and are associated with nearly all aspects of animals' life-history. In this thesis I had two main objectives. First, using phylogenetic comparative methods, field observations and laboratory experiments, I explored three key functional hypotheses of SSD (sexual selection, fecundity selection and differential niche-utilisation) using odonates, seabirds and bustards as model organisms. My results are most consistent with sexual selection promoting changes in males toward small or large size, depending on the intensity of sexual selection and on the form of male-male competition. My second objective was to investigate how SSD develops during larval development and emergence to maturity in odonates. Therefore I first tested the influence of food supplementation during larval stages on adult body sizes in American Rubyspots. Supplementary feeding, however, influenced neither the size nor the SSD of emerged adults. Finally, I explored the development of SSD from last larval stage into adulthood using seven species of Odonata. I show that SSD in some species is already evident from the larval stage; nevertheless, as a general rule, the direction of adult SSD is not predictable from the SSD of larvae.

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Chapter 1. *Sexual size dimorphism in damselflies, dragonflies and birds: function and development*

M. Alejandro Serrano-Meneses

1. INTRODUCTION

A common observation in many animal species is that males and females exhibit differences in their body sizes. Some of the sexual differences in size (sexual size dimorphism; SSD) are dramatic, for example male Northern Elephant Seals (*Mirounga angustirostris*) can be up to five times heavier than females (Andersson 1994), whereas Blanket Octopus (*Tremoctopus violaceus*) females can be up to 40,000 times heavier than males (Norman et al. 2002). This extraordinary variation in SSD has attracted the attention of biologists, and inspired several hypotheses to explain the variation in size across animal species ever since Darwin's (1871) seminal exposure of SSD (reviewed by Jehl & Murray 1986; Hedrick & Temeles 1989; Shine 1989; Andersson 1994; Blanckenhorn 2005). To date, Darwin's (1871) original ideas on the evolution of SSD form the foundation of modern research on SSD. Nevertheless, the explanatory power of these ideas in a particular species or across a range of organisms has remained controversial (Blanckenhorn 2005).

Body size is usually correlated with a number of physiological, fitness traits and mating advantages (Fairbairn et al. 2005). Males and females are genetically nearly identical, yet, they sometimes exhibit extreme differences in size (see above). Therefore, since SSD affects and is affected by animal life-histories, behaviour, development and ecology, the understanding of the processes that cause SSD in animals provides a unique research opportunity to understand how evolution works (Fairbairn et al. 2005). That is, the study of SSD provides visible and testable evidence of the effect of natural and sexual selection and their capabilities to cause differentiation between the sexes, even under the influence of strong developmental constraints (reviewed by Blanckenhorn 2005; Fairbairn et al. 2005).

To date, few SSD studies cross the boundaries between the disciplines of evolutionary biology. For instance, researchers usually adopt a single perspective when investigating SSD (i.e. quantitative genetics, phylogenetic comparative methods or development). This is not ideal since our understanding of the processes that select for SSD is not

enriched from cross-disciplinary feedback, resulting in fragments of knowledge that lack integration. Another limitation in the study of SSD is that empirical and theoretical research are usually carried out in isolation from each other (Blanckenhorn 2005). Therefore, in this thesis, I adopt a multidisciplinary approach in my study of the processes that select for SSD and I combine comparative phylogenetic analyses with ecological and behavioural study of a single species in the field. This integrative approach provided me a better understanding of the evolution of SSD, and highlighted some of the problems that may blemish narrower approaches (see Discussion).

Why are there differences in size between the sexes? Three major groups of functional hypotheses were put forward to explain the selective processes separating the sizes of the sexes: sexual selection, differential niche-utilisation (or ecological division of resources), and fecundity selection (Darwin 1871; Andersson & Norberg 1981; Jehl & Murray 1986; Hedrick & Temeles 1989; Shine 1988, 1989; Andersson 1994; Blanckenhorn 2005). Studies on SSD usually test these hypotheses in fully grown animals using the comparative and/or behavioural ecological approach (Cox et al. in prep.; Lindenfors et al. in prep.; Székely et al. in prep.), whereas the development of body size (hence SSD) throughout the ontogeny of individuals is less commonly investigated (Badyaev 2002; John-Alder & Cox in prep.; Kalmbach & Benito in prep.).

The major objectives of my PhD were (i) to test functional hypotheses of SSD in Odonates (damselflies and dragonflies) and birds (seabirds and bustards), and (ii) to investigate the development of SSD from larvae to tenerals in Odonates. This somehow unusual combination of study organisms was a necessity driven by the less amenable nature of birds to functional and development tests of SSD than I initially envisaged.

On the one hand, to investigate the functional hypotheses of SSD I used phylogenetic comparative methods in both odonates and birds. In addition, I carried out fieldwork to investigate the advantages of male body size in a territorial tropical damselfly (American Rubyspot, *Hetaerina americana*) in behavioural observations. There are two justifications for using a combination of these approaches. Firstly, comparative studies

often expand the horizon of single-species studies, especially if the trait in question is not readily amenable to experimental manipulation, such as SSD. Secondly, during the PhD I had a chance to develop skills in regards to both field ecology and phylogenetic comparative analyses. On the other hand, to investigate the development of SSD I carried out a food supplementation experiment in American Rubyspots to induce changes in their body size and SSD. Also, I reared seven species of odonates in laboratory in order to determine whether SSD arises during the development or at maturity.

Below, first I outline the theoretical background of SSD and then discuss why odonates, seabirds and bustards are suitable taxa for testing functional hypotheses of SSD. I then discuss the specific objectives and novel results of each chapter. Finally, I propose potential research avenues for future studies of SSD and sexual dimorphism.

2. BACKGROUND

*'With insects of all kinds the males are commonly smaller than the females; and this difference can often be detected even in the larval stage. So considerable is the difference between the male and female cocoons of the silk-moth (*Bombix mori*), that in France they are separated by a particular weighing.'*
Charles Darwin, 1871 (p 320)

2.1 Sexual size dimorphism

Darwin (1871) noted that the sexes usually differ in size in several animal species. He proposed that these differences were largely due to fecundity selection acting on female size, and sexual selection acting on male size. Therefore, under fecundity selection, females are expected to be the larger sex, whereas males will be larger than females if large size grants males with a mating advantage. These ideas are now supported by a number of studies (reviewed by Andersson 1994, Blanckenhorn 2005, Fairbairn et al. in prep.). Nevertheless, fecundity selection and sexual selection are unlikely to be the only selective pressures shaping SSD across animal taxa. Thus, body size is likely to be influenced and/or constrained by natural selection (reviewed by Shine 1989), as it occurs

with other morphological traits (e.g. Temeles et al. 2000). Note that both fecundity- and sexual selection are expected to promote increases in body sizes of females and males, respectively (Andersson 1994). Therefore, these selective pressures will tend to drive the sizes of the sexes away from their optimum (larger than the optimum size favoured by natural selection), sometimes compromising an individual's survival (i.e. through the increased costs of supporting large size and foraging time). Usually, selection for increased body size will be opposed by viability selection (Blanckenhorn 2000), which will determine optimal growth, age and size at maturity (Roff 1980, 1992; Stearns & Koella 1986; Kozlowski 1992; Stearns 1992). The interplay between different selective pressures acting on males and females will determine SSD within a species (Figure 1).

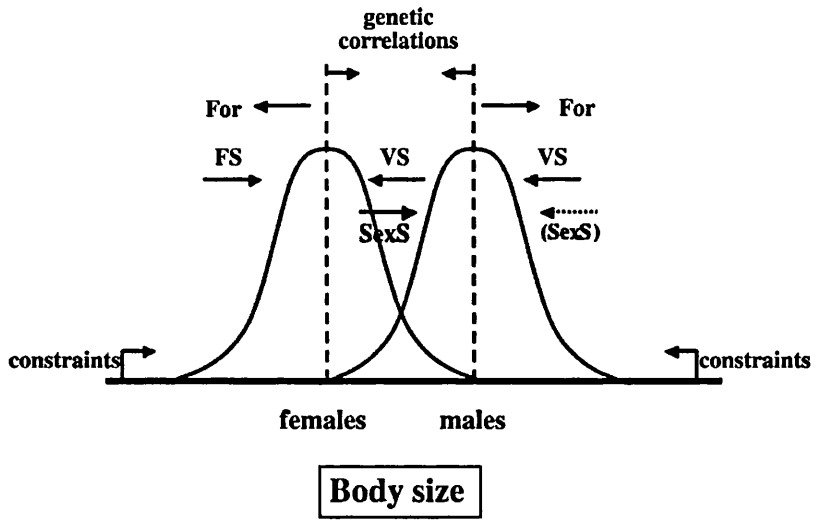

Figure 1: The differential equilibrium model of sexual size dimorphism (SSD). Body size distributions are shown for a population in which males are larger on average than females. Fecundity selection (FS) tends to select for increased body size in females, and sexual selection (SexS) for increased body size in males (but occasionally in the opposite direction). Viability selection (VS) select for smaller body size in both sexes. Foraging (For) specialization may select for divergent body sizes of males and females. Constraints and genetic correlations between the sexes are also shown that may limit the expression of SSD (after Blanckenhorn 2005).

How do organisms achieve large size? There are two fundamental ways: (i) organisms may grow for longer periods, or (ii) they may grow faster (Blanckenhorn 2000; Badyaev 2002). In the first case, organisms may go through an extended pre-reproductive period in which mortality rates are high due to predation, parasitism or starvation. When

animals grow faster to achieve large size, then mortality will be increased through the higher metabolic costs of being large, and exposure to predators due to the increased foraging activity necessary to achieve large size (Blanckenhorn 2000). Therefore, there are trade-offs in the development of large size (Partridge & Fowler 1993; Arendt 1997; Blanckenhorn 2000), since large size is also associated with an advantage in mating (Andersson 1994). Should organisms develop faster and breed at smaller sizes? Or should organisms grow for longer and achieve large size but compromise potential mating opportunities? These trade-offs and other environmental factors (such as temperature; Atkinson 1994) are likely to determine adult body size.

Is bigger body size always better than a small body size? Not necessarily. The selective advantage(s) of a given body size may depend on a number of factors. Table 1 lists a number of selective factors that likely to influence the degree and direction of SSD, through the advantage of a given body size (Andersson 1994). The factors listed in Table 1, however, fall into a number of functional hypotheses of SSD. In the following sections I explain these hypotheses.

Table 1. Selective factors that may influence the sizes of sexes, and thus the direction and degree of sexual size dimorphism (from Andersson 1994).

Female advantages of large size: Higher fecundity; better parental care; male preferences for large females; dominance in contests over resources, or over males in role-reversed species.
Female advantages of small size: Earlier maturation, with shorter generation time and more rapid reproduction as conditions become favorable; more effective shunting of resources into offspring production
Male advantages of large size: Dominance in contests over females or resources when strength is crucial; better performance in endurance rivalry; female preferences for large males; higher success in sperm competition
Male advantages of small size: Dominance in contests over resources when maneuverability rather than strength is crucial; earlier maturation, with more rapid reproduction and shorter generation time; higher success in scrambles; more surplus energy available in searching for mates; female preferences for small males

2.1.1 Sexual selection

Darwin (1871) proposed that certain individuals have reproductive advantages over other individuals of the same sex, thus, when the competition between individuals of the

same sex is more intense than between the individuals of the other sex (usually the males), sexual selection will drive the size of the more competitive sex towards an advantageous optimum size (Székely et al. 2000). There are, therefore, two ways in which sexual selection may influence SSD. First, the optimum size of the competing sex may depend on whether the competition occurs on the ground or in the air (Payne 1984; Jehl & Murray 1986; Figuerola 1999; Székely et al. 2000; Székely et al. in prep). Therefore, sexual selection is expected to favour large males relative to females (i.e. male-biased SSD) when sheer bulk increases the chances of winning, since the power of a blow increases with muscle size (Clutton-Brock & Harvey 1977). Sexual selection, however, will favour small male size relative to females (female-biased SSD), if agility and manouverability enhance the males' success such as in aerial combats (Andersson & Norberg, 1981). Small male size may be reinforced by female preference for small, agile males (Grønstøl 1996; Hakkarainen et al. 1996; Blomqvist et al. 1997; Figuerola 1999). This likely to occur in many shorebirds (Székely et al. 2000, 2004) in which evolutionary increases in male agility are correlated with evolutionary changes toward female-biased SSD.

Second, sexual selection (via male-male competition) may favour large male body size in species in which males compete intensely over females (Clutton-Brock & Harvey 1977; Mitani et al. 1996; Owens & Hartley 1998; Dunn et al. 2001; Lindenfors et al. 2003; Lindenfors et al. in prep.; Székely et al in prep.). Thus, large size is advantageous to males in species with polygynous mating systems (Clutton-Brock & Harvey 1977; Owens & Hartley 1998). This is likely to be the case if larger-than-average males are able to endure competition for longer than small males, and if they are preferred by females, for instance, because these males may offer better resources for the females. Large size also favours males to achieve forced copulations (reviewed by Andersson 1994).

2.1.2 Fecundity selection

Darwin (1871) also suggested that large size may be favoured in females by increasing their fecundity, especially if body cavity limits the number of eggs a female can bear.

Therefore, female fecundity is expected to favour female-biased SSD, which is commonly exhibited by many invertebrates, fishes and birds (Andersson 1994; Blanckenhorn 2005). This likely to occur if large females achieve higher reproductive success through higher capacity for producing and laying eggs (Ridley & Thompson 1979; Wiewandt 1982; Cordero-Rivera 1991; Honěk 1993). Consistently with fecundity selection, studies of spiders, insects and ectothermic vertebrates (fish, frogs), demonstrated positive relationships between body size and fecundity (Andersson 1994; Head 1995; Preziosi et al. 1996; Prenter et al. 1999; Legaspi & Legaspi 2005).

Fecundity selection may also favour large females if they provide better parental care that enhances offspring survival (Wauters & Dhondt 1995; Ralls 1976).

2.1.3 Differential niche-utilisation

Selection may act on body sizes of males and females simultaneously to avoid competition with each other, or to enhance prey catching when resources are scarce (Selander 1966; Selander 1972; Shine 1989). Thus specialisation in the exploitation of resources is expected to lead to morphological divergence. For example, male and female Purple-throated Carib Hummingbirds (*Eulampis jugularis*) feed specifically from the flowers of *Heliconia caribaea* and *H. bihai*, respectively. This specialisation has lead to a morphological divergence in culmen size and shape between the sexes: each sex's culmen is correlated to the length and curvature of the flowering plant from which they feed (Temeles et al. 2000). Therefore body size, in a similar fashion, was proposed to adapt to a differential resource utilisation between the sexes (Selander 1966; Shine 1989; Sandercock 2001), and enable the members of a dimorphic pair, for instance, to exploit a wider ecological niche than monomorphic pairs (Figuerola 1999).

This hypothesis, however, fails to predict the direction and degree of SSD: either males or females can be the larger sex (Székely et al. 2000). In addition, it is not clear whether different resource use by males and females drove SSD, or SSD evolved for a different reason and as a consequence of different body sizes males and females are exploiting different resources.

2.2 Rensch's rule

'This rule, however, applies only to subspecies of a species, to related species of a genus, or to related genera of a family.' Bernhard Rensch, 1959 (p 159)

Across species of a particular taxon, males and females often show a puzzling relationship between SSD and body size: SSD increases with body size in species in which males are larger than females, and it decreases with body size in species in which females are larger than males (Rensch 1950; Fairbairn 1997; Figure 2).

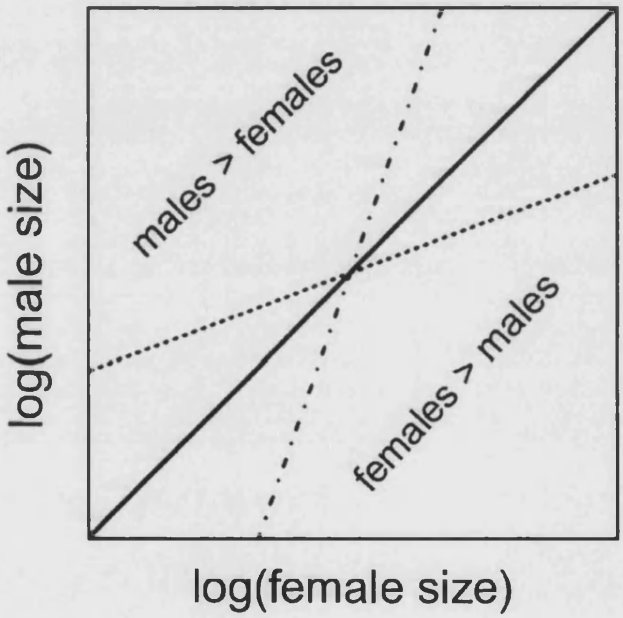

Figure 2. Rensch's rule. The *solid line* represents the isometric relationship between the size of males and females (size of males = size of females). The *dotted line* represents an allometric relationship in which female size varies more among species than male size, and therefore the regression slope (β) is less than one. The *dot-dashed line* represents an allometric relationship in which male size varies more among species than female size and therefore $\beta > 1$. Deviations of these lines from the 1:1 line illustrate the degree of SSD. When $\beta > 1$ SSD declines as size increases for female-biased species, but increases with size for male-biased species, as predicted by Rensch's rule. If $\beta < 1$, the pattern of allometry is reversed, and is inconsistent with the Rensch's rule (adapted from Fairbairn 1997).

This pattern is exhibited by a wide range of animal taxa that includes mammals (Ford 1994; Abouheif & Fairbairn 1997), birds (Payne 1984; Björklund 1990; Webster 1992; Colwell 2000; Székely et al. in prep.), reptiles (Abouheif & Fairbairn 1997) and insects (Sivinski & Dodson 1992; Colwell 2000). Not surprisingly, a number of functional

hypotheses have been proposed to explain Rensch's rule for specific taxa (reviewed by Reiss 1986; Webster 1992), nevertheless, it is generally agreed that sexual selection acting on male size is likely to account for the observed allometric pattern (Abouheif & Fairbairn 1997). On the one hand, sexual selection likely to promote increases in male body size that will be followed by small increases in female size due to the genetic correlation between the sexes, explaining the increasing extent of SSD in large species. On the other hand, sexual selection, in a similar fashion, may favour small male size and hence the reduction of male size over time that is followed by decreases in female size. Therefore, male body size is assumed to change more rapidly than female size, for instance because female size may be under fecundity selection pressure (Abouheif & Fairbairn 1997).

This hypothesis has been tested recently in shorebirds (plovers, sandpipers and allies, Székely et al. 2004) that show the full scope of Rensch's rule. This comparative work showed that Rensch's rule emerged in shorebirds as a combined result of selections emerging from male-male competition and the agility of male displays. To date, Székely et al. (2004) is the only comparative study that related the Rensch's rule directly to the influences of sexual selection.

3. DISTRIBUTION OF SSD IN ODONATES, SEABIRDS AND BUSTARDS

I chose to work on odonates, seabirds and bustards because these groups fulfilled a number of criteria. First, testing functional hypotheses of SSD, requires groups that exhibit natural variation in both SSD and putative traits such as mating systems, male display agility and resource use. Information on body sizes, behaviour and ecology should be accessible in handbooks and other published sources for many species; indeed, this is the case for these three taxa. Second, a robust phylogenetic hypothesis should be available for many species. This is very important since phylogenetic comparative methods assume that the phylogenetic hypotheses are reliable. Third, both odonates and birds have determined growth: adults do not continue growing after they reach sexual

maturity. This avoids the problem of age-dependent SSD (Monnet and Cherry 2002). Finally, the use of these groups allowed me to test the general applicability of the functional theories of SSD, and to use a multidisciplinary approach in order to understand SSD.

3.1 Damselflies and dragonflies

'So again, male dragon-flies (Libellulidae) are sometimes sensibly larger, and never smaller, than the females...' Charles Darwin, 1871 (p 321)

The order Odonata (damselflies and dragonflies) comprises approximately 6000 species of global distribution (Corbet 1999). Odonate species have a wide range of behaviour, habitats, morphology and mating tactics, that include territorial defence and scramble competition (reviewed by Corbet 1999; Fincke et al. 1997). Odonates exhibit both male- and female-biased SSDs (Kruskal-Wallis H = 48.939, p = 0.0001; Figure 3): males are larger on average than females, for instance, in Calopterygidae (Wilcoxon one-sample test W = 648, p = 0.0001), whereas females are larger in Coenagrionidae (W = 84, p = 0.034). Note that the latter fact appeared to avoid the attention of Charles Darwin - this is unusual, given Darwin's outstanding knowledge of natural history. A range of SSD may be observed within a single family (i.e. Gomphidae; W = 49, p = 0.839).

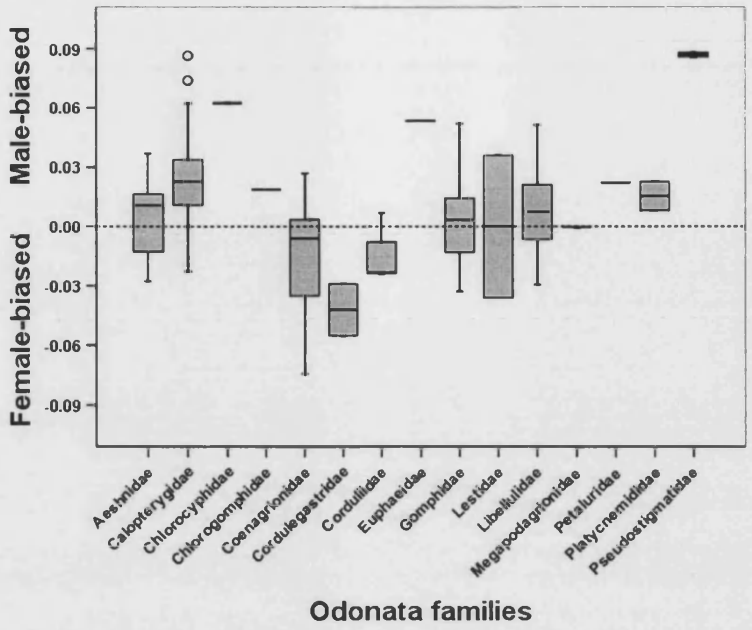

Figure 3. Sexual size dimorphism in Odonata families (median, upper and lower quartiles; open circles are extremes). SSD = log_{10}(male body length) — log_{10}(female body length). The dotted line denotes monomorphism. The number of species is 7, 36, 1, 1, 25, 2, 4, 1, 13, 2, 35, 1, 1, 2, and 2 in each family, from left to right. See Chapter 5 for the dataset.

SSD has been studied only in few odonates. For instance, in a comparative study, Anholt et al. (1991) analysed the patterns of mass gain in several odonate species. In that study, Anholt et al. (1991) showed that females were usually heavier than males across odonates; however, this difference was less evident and even the opposite in territorial species. It was then assumed that sexual selection, acting on male size, was driving the observed patterns of SSD in territorial and non-territorial species (Anholt et al. 1991; reviewed by Andersson 1994). Nevertheless, the prediction that territorial odonates would generally exhibit male-biased SSD is not always met (reviewed by Fincke et al. 1997). For example, whilst large male size is advantageous in some territorial species (i.e. Fincke 1984; Tsubaki & Ono 1987), small males are more successful in others (i.e. Convey 1989). Therefore the influence of sexual selection on the SSD of odonates has been debated and questioned (Fincke et al. 1997; Sokolovska et al. 2000; Thompson & Fincke 2002), so that currently there is no agreement as to what drives SSD in odonates (Fincke et al. 1997; Andersson 1994; Sokolovska et al. 2000).

More recently, Johansson et al. (2005) investigated Rensch's rule in Odonata using phylogenetic independent contrasts (Felsenstein 1985). Nevertheless, their study was limited to 21 species (15 anisopterans, 6 zygopterans) and they only carried out a limited test of functional hypotheses (see below).

3.2 Seabirds

Seabirds are a paraphyletic group of birds that make a living from marine environments (Schreiber & Burger 2002). Seabirds comprise approximately 190 globally distributed species (Monroe & Sibley 1993; Spheniscidae (Penguins), Phaethontidae (Tropicbirds), Pelecanidae (Pelicans), Sulidae (Gannets and Boobies), Phalacrocoracidae (Cormorants), Fregatidae (Frigatebirds), Diomedeidae (Albatrosses), Procellariidae (Petrels and Shearwaters), Hydrobatidae (Storm-Petrels) and Pelecanoididae (Diving-Petrels)). Seabirds show a wide range of foraging strategies, geographic distribution, social organization and SSD (see Chapter 6). Also, seabirds exhibit a range of male displays; from ground displays by Cormorants to the aerial acrobatic display of some Storm-Petrels (Marchant & Higgins 1990). Finally, it is possible to calculate the ocean primary productivity of the areas where these birds breed. This group is particularly interesting because all species are socially monogamous, both sexes invest heavily in parental care and the sexes are similar in external appearance (Fairbairn & Shine 1993).

A previous comparative study on the evolution of SSD in seabirds by Fairbairn & Shine (1993) found that seabirds exhibit Rensch's rule, that the frequency distribution of SSD in seabirds is generally male-biased, and that larger species live in areas of higher sea productivity. Nevertheless, Fairbairn & Shine's (1993) study had a number of limitations (see Chapter 6). For example, their study only included Southern Hemisphere species and the effect of sexual selection on SSD was not tested, presumably because the intensity of sexual selection was thought to be low, due to prevailing social monogamy in this group. We therefore tested how sexual selection may influence SSD of seabirds.

3.3 Bustards

Bustards (Otididae) comprise 25 species of wide distribution in Europe, Africa, Eurasia and Australia (Johnsgard 1991). Bustards are an excellent group to investigate SSD because they exhibit a wide range of habitats, and a full range of SSD that includes both male- and female-biased SSD. For example, adult male Great Bustards (*Otis tarda*) can be up to three times as heavy as females, whereas male Lesser Floricans are smaller than females (Dale 1992). Bustards exhibit mating systems that range from social monogamy to lek breeding, and diverse courtship displays. For example, male Great Bustards show sophisticated ground-displays to females in which the males inflate the gular pouch, and ruffle their feathers (balloon display). As a result, a displaying male appears to be much bigger than a non-displaying one. In contrasts, male Bengal Floricans (*Eupodotis bengalensis*) spring up vertically with loud wing-flapping and reaches heights of up to 4 metres (Johnsgard 1991). In addition to these diverse behaviours, the phylogenetic relationships of bustards are well understood (Pitra et al. 2002) making of this group a promising research avenue for testing ideas on the evolution of SSD.

4. TESTING FUNCTIONAL HYPOTHESES OF SSD

4.1 Sexual selection and male body size in American Rubyspot

The American Rubyspot damselfly (*Hetaerina americana*) was the subject of studies on male wing pigmentation (Grether 1996a, 1996b, 1997; Grether & Grey 1996; Contreras-Garduño et al. 2006). Grether (i.e. 1996a, 1996b) for instance, focussed on the red spot the males exhibit at the base of each wing, and Grether provided evidence that these spots are sexually selected *via* male-male contests. Nevertheless, the costs and benefits of large size for males had not been directly investigated prior to my thesis (*Chapter 2*).

The American Rubyspot is a sexually dimorphic zygopteran that exhibits male-biased SSD. Males of this species, similarly to other territorial odonates (Corbet 1999), attempt to establish and defend territories along streams and rivers (Johnson 1963), and females are attracted to these areas for copulation or oviposition (Corbet 1999; Córdoba-Aguilar

& Cordero Rivera 2005). Therefore, territory tenancy is expected to influence mating success in territorial odonates (Corbet 1999). Not all males are able to establish territories, and consequently, mating success is expected to differ between territorial and non-territorial males: territorial males usually more successful, in terms of mating success, than non-territorial males (i.e. Córdoba-Aguilar 1995; Grether 1996a; Plaistow & Siva-Jothy 1996; Córdoba-Aguilar & Cordero-Rivera 2005). Therefore, I investigated whether body size may relate to territoriality and mating success, and to other morphological characters such as wing pigmentation and energy reserves (*Chapter 2*).

First, I show that large males are better at holding territories (i.e. they are able to sustain longer fights and hold the territories for longer), and that they obtain more copulations than non-territorial males. Second, energy reserves and wing pigmentation (i.e. male sexual ornament) are positively correlated with male body size. Third, selection analyses of body size show disruptive selection, which suggests that both large and small males may be favoured in terms of mating success. Interestingly, similar to other studies (i.e. Bürger & Schneider 2006) disruptive selection on male size is accompanied by assortative mating by size. Fourth, my study showed no relationship between female body size and fecundity, since large females did not carry more or larger eggs than small females.

These results support the hypothesis that large size is advantageous for males in a territorial damselfly species *via* male-male competition, and territory acquisition and/or maintenance. My results also suggest that small size may be advantageous to no-territorial males since it probably improves their agility in courting (or subduing) females.

4.2 Comparative analyses of SSD in Odonata: Rensch's rule and sexual selection

Using phylogenetic independent contrasts (Felsenstein 1985) I show that odonates exhibit Rensch's rule, however, this relationship depends on the inclusion of both Anisoptera and Zygoptera in the analysis (*Chapter 5*). Once these groups were analysed separately, only Zygoptera showed an allometry consistent with Rensch's rule.

I also tested the influence of sexual selection (as estimated from territoriality, non-territoriality and male agility) on SSD using generalised least squares (Pagel 1997, 1999; Garland & Ives 2000; Freckleton et al. 2002). I found that evolutionary increases in male-biased SSD correlated with evolutionary increases in territoriality in Zygoptera, but not in Anisoptera only. Male agility (as estimated by male wing asymmetry) was unrelated to SSD.

What may explain Rensch's rule in odonates? I tested whether sexual selection, using proxy variables of territoriality and male agility, correlate with the allometric relationship between the sizes of males and females. My results show that, although sexual selection in the form of territoriality and non-territoriality contributes to the observed pattern, it is not the sole selective pressure that influences the Rensch's rule.

4.3 SSD in seabirds: sexual selection, fertility selection and differential niche-utilisation

In *Chapter 6* I tested three functional hypotheses of SSD: sexual selection, fecundity selection and differential niche-utilisation. Despite most seabirds being socially monogamous, my results supported the sexual selection hypothesis, because evolutionary changes towards agile male displays were correlated with evolutionary increases in female-biased SSD. I found no support for the fecundity hypothesis, since clutch size was not associated with increases in female-biased SSD. Finally, contrary to Fairbairn & Shine's (1993) study, my results do not support the differential niche-utilisation hypothesis, since larger dimorphisms did not occur in areas of low ocean productivity. The discrepancy between the conclusions of Fairbairn & Shine (1993) and my study may have two reasons. First, we argue that Fairbairn and Shine's own data do not support their conclusions. Second, differences in ocean productivity data may have lead to divergent conclusions. Fairbairn & Shine (1993) estimated the minimum levels of rates of primary productivity surrounding the breeding areas as the rate of carbon fixation (g m^{-2}) from a global map with 4 possible levels of productivity. In contrast, I calculated ocean productivity from a map that estimated chlorophyll concentration

(grams of carbon m^{-2}) from visible light reflectance. This map had approximately 450 levels of productivity (see *Chapter 6*).

I conclude that SSD in seabirds is most consistent with the sexual selection hypothesis. This study also highlights the need for sex specific data on feeding strategies and the influence of within-pair SSD on breeding success.

4.4 SSD in bustards: sexual selection and male agility

Bustards exhibit an allometry consistent with Rensch's rule (*Chapter 7*), and this relationship is one of the strongest allometric relationships in any avian taxa. I also tested whether sexual competition and male display agility may correlate with SSD (Figure 4). Using a multiple regression model of phylogenetically independent contrasts, I show that both mating system and male display agility are significant predictors of SSD when controlling for each other's influence.

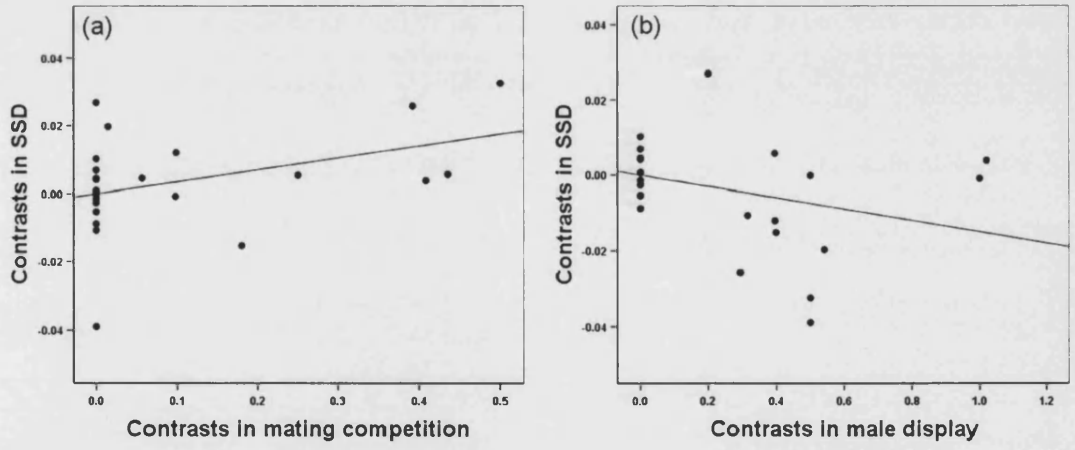

Figure 4. Phylogenetically independent contrasts in sexual size dimorphism (SSD) and (a) the intensity of mating competition ($r = 0.453$, $F_{1,23} = 5.934$, $P = 0.023$) and (b) male agility ($r = 0.409$, $F_{1,23} = 4.634$, $P = 0.042$). Evolutionary increases in mating competition are correlated with evolutionary increases in male-biased SSD, whereas increases in male display agility are correlated with increasing female-biased SSD.

Unlike seabirds, which are socially monogamous, bustards exhibit Rensch's rule, whereas seabirds do not. I suggest that the different intensity of sexual competition

between these two groups may explain why one group shows an allometric relationship, whereas the other does not.

5. THE DEVELOPMENT OF SSD

5.1 Larval diet and the development of SSD in American Rubyspots

Adult body size is usually determined by genetic, environmental and/or maternal factors (Blanckenhorn 2000, Badyaev 2002). In an experimental study I investigated the effect of larval diet on the development of SSD in both larvae and adult American Rubyspots (*Chapter 3*). I collected larvae from their natural environments in Morelos, Mexico, and subjected them to three different diets: poor, intermediate and rich. The treatment, however, did not influence adult body size and hence SSD. There may be four reasons for this. First, food alone may not be the determinant of adult body size, but instead, the result of a more complex interplay between genetic and other environmental variables. Second, the effect of diet regimes may be more evident in developmental time, rather than body size alone. This is possible since odonates do not have a fixed number of instars and they are likely to change with changing environmental variables. Third, it is possible that our larvae samples included individuals that had undergone most of their growth and as a consequence were not affected by the treatments. Finally, mortality was high which reduced sample size making it less likely to find statistical difference, even if this exists (Type II error).

5.2 The development of sexual differences in body size in Odonata

Adult SSD may simply be a consequence of size dimorphism already existing during early development. Alternatively, dimorphism in adults may develop when the teneral develops from sexually monomorphic larvae. I tested these alternatives in *Chapter 4* using seven species of Odonata (*Anax imperator, Cercion lindeni, Cordulegaster boltonii, Ischnura graellsii, Onychogomphus uncatus, Oxygastra curtisii* and *Platycnemis acutipennis*). Body sizes of larvae were measured at the last larval stage in order to estimate the degree and direction of SSD. Then the larvae were reared in the

laboratory, and the emerging adults were also measured. The results show that SSD consistent with adult SSD was already exhibited in the larval stage in *Anax imperator, Cercion lindeni, Cordulegaster boltonii* and *Platycnemis acutipennis*, whereas in *Onychogomphus uncatus* adult dimorphism emerged from monomorphic larvae. These results suggest that developmental pathways may be different between closely related odonates that influence growth and metamorphosis, and ultimately, adult sizes.

6. CONCLUSIONS

I have shown that sexual selection, *via* mating competition and/or male display agility correlates with SSD in damselflies, seabirds and bustards. This result is somehow surprising, since some of these groups, notably seabirds, were thought to be subject to weak (or no) sexual selection. These processes, however, are presumed to act only on males. This assumption, however, may not be fully correct, because females may also compete over mates (Slagsvold & Lifjeld 1994; Liker & Székely 1997). This implies that the same processes proposed to influence male size can in principle influence female body size. Nevertheless, no selection for female body size in the context of sexual selection has been demonstrated (Blanckenhorn 2005).

I have shown that sexual selection is generally a good predictor of the direction and degree of SSD in these taxonomic groups. For instance, my results in seabird and bustard SSD do not only show that sexual selection has the potential to promote changes towards male-biased SSD, but they also show that male-male competition (perhaps reinforced by female choice) can also favour small male size. These results are novel and fall in an open area in the research of SSD, since there are already many studies supporting the advantages of large male size, whereas few studies have ever demonstrated the disadvantages of large male body size (reviewed by Blanckenhorn 2005). This is also the case in SSD of odonates (*Chapter 5*); nevertheless, this study (*Chapter* 5) has other important implications. For instance, this is the first study, to my knowledge, to use phylogenetic comparative methods to test a functional hypothesis of

SSD (sexual selection) in odonates. Also, the results that I obtained point out why previous researchers on odonate SSD did not achieve an agreement on the effect of sexual selection on SSD. Whilst some researchers proposed that large male size was advantageous for males of odonate territorial species (i.e. Anholt et al. 1991; Sokolovska et al. 2000), others found no relationship between territoriality and SSD (i.e. Fincke et al. 1997). My results thus suggest that the expected relationship between territoriality and SSD (increasing male-biased SSD with increasing territoriality) is only exhibited by damselflies (Zygoptera), but not by dragonflies (Anisoptera). This is noteworthy, since it suggests marked differences in the effects of sexual selection on the morphology and behaviour between these sub-orders.

The effect of sexual selection on SSD is such, that it is capable of leading to macroecological trends in body size between the sexes (see below). An allometric relationship consistent with Rensch's rule is exhibited by damselflies and bustards, it is marginally not significant in seabirds and it is not exhibited by dragonflies. Interestingly, in the taxonomic groups that exhibit this allometric trend, sexual selection correlates with SSD (see also Székely et al. 2004). Although seabird SSD is correlated with sexual selection, the effect is weaker than it is in bustards and damselflies, which provides a possible explanation to the marginally non-significant allometric trend in seabirds. My results suggest that, contrary to what occurs in shorebirds (Székely et al. 2004), in Odonata sexual selection is unlikely to be the only explanation of Rensch's rule.

A limitation of these comparative studies, however, is that one can only use those data that were collected across a broad range of species and this restricts detailed analyses of behaviour and ecology. Therefore, I designed an empirical study dedicated to evaluate the processes behind SSD in a single species study. My behavioural approach on the study of SSD in the American Rubyspot produced results that are consistent with the idea that male-biased SSD is maintained (or even promoted) by sexual selection in this species. In this study I also quantified the selection acting on male size. Measuring selection is useful since it generates data that can later be used for meta-analyses and other systematic comparative studies. Nevertheless, researches of SSD rarely calculate

selection differentials (reviewed by Blanckenhorn 2005). Therefore, by measuring the selection acting on male body size I established that disruptive selection acts on body sizes of male American Rubyspots. Note that this was accompanied by assortative mating by size, since large males mated with large females and small males mated with small females. To my knowledge, this is the first study to document assortative mating by size accompanied by disruptive selection acting on body size in odonates. This suggests that although large male size confers 'instantaneous' mating advantages to territorial males, small males overcome the disadvantages of small size and achieve a number of copulations.

Alternative male mating strategies are commonly found in territorial odonates (Corbet 1999); however, only few species show marked morphological differences between the territorial and the alternative mating strategies. In such cases, the morphological differences are so conspicuous that they are denominated morphs (Corbet 1999). For example, territorial 'fighter' males of the Japanese Damselfly (*Mnais pruinosa*) are orange-winged and significantly larger than the clear-winged, small, non-territorial 'sneak' males (Tsubaki et al. 1997). Orange-winged males establish and defend territories, achieving a greater number of copulations than the clear-winged males and in a short time span (Tsubaki et al. 1997). Interestingly, in a few populations the orange-winged males are absent, and clear-winged males exhibit a range of mating strategies that includes territorial, non-territorial and opportunistic males (Siva-Jothy & Tsubaki 1989a, 1989b).

Why are there different mating strategies and male morphs? The male dimorphism observed in the Japanese damselfly is thought to be genetically determined (Tsubaki et al. 1997) and driven by a trade-off between reproductive advantages and longevity, underpinned by differences in energy expenditure between the morphs (Plaistow & Tsubaki 2000). This is because territoriality in damselflies is usually determined by the amount of body fat reserves, which are the main source of energy during territorial contests (i.e. Marden & Waage 1990; Plaistow & Siva-Jothy 1996) and they are limited by body size (i.e. Anholt et al. 1991). Therefore, since not all males attain large size and

high energy reserves, alternative mating strategies are adopted by those males bearing the disadvantage of small size and low energy reserves. I have shown this is the case in the male-monomorphic American Rubyspot. Therefore, I suggest that the observed male dimorphism in the Japanese damselfly and other species, such as *Paraphlebia quinta* (González-Soriano & Córdoba-Aguilar 2003), may be the result of disruptive selection acting on the traits that confer a mating advantage. Regretfully, no study has measured the selection acting on male size in such species, but it is known that large size is associated with mating success in orange-winged males and that it confers no mating advantages to clear winged males (Plaistow & Tsubaki 2000). Therefore, selection on body size is inferred to differ between morphs (Plaistow & Tsubaki 2000). Note that these species are polygamous (Corbet 1999), which provides opportunities for speciation and the diversification of traits in the face of high levels of sexual selection (i.e. Arnqvist et al. 2000).

Why do not all animals achieve large size? Large size is not achieved without costs (Blanckenhorn 2000; Badyaev 2002). In insects, large body size is usually associated with long developmental time (Roff 1992), therefore, marked mating seasons may impose pressure on males to emerge early in the season. If males delay development for too long, they may pay significantly fitness costs (Rowe et al. 1994; Johansson & Rowe 1999; Plaistow & Siva-Jothy 1999). This will impose trade-offs between developmental time and body size and selection will be favouring the body size that maximises fitness (Plaistow & Siva-Jothy 1999). This will depend on the influence that body size has on mating success, as well as the time and resources available for development (Johansson & Rowe 1999; Plaistow & Siva-Jothy 1999). In odonate species with marked seasonality, for instance, the direction of adult SSD can sometimes be defined during the larval stage (*Chapter 4*), suggesting the presence of pressure to meet the emergence deadline set by seasonality. Nevertheless, in species with no marked seasonality, this pressure should be more relaxed, allowing individuals to remain as a larvae for longer in order to accumulate the resources necessary to achieve large size. Although I did not find evidence for the effect of larval diet on the development of adult body size in the American Rubyspot (*Chapter 3*), my results show that this species exhibits a non-

significant trend which suggests that individuals may respond to high resource availability by developing for longer. Taken together, my results on the development of SSD support the findings of my comparative work on odonate SSD (*Chapter 5*): large male size is more likely to develop in territorial than in non-territorial damselfly species. Large size is also less likely to develop in territorial odonates.

In conclusion, my interdisciplinary and taxon-diverse approach on the study of SSD revealed patterns of SSD and macroecological trends that are likely to be caused by variation in the selective pressures acting on adults and on the development of males and females.

7. FUTURE DIRECTIONS

Taken together, the research presented herein provided new insights into the role of sexual selection as a general evolutionary mechanism, and expected to advance our understanding of the diversity of SSD in seabirds, bustards and odonates. Nevertheless, there are a number of issues future works should address.

First, it is not clear whether male-male competition, female choice or both have an influence on SSD. Whereas the amount of evidence showing the advantages of large male size is outstanding (Blanckenhorn 2005), only a few studies have shown that sexual selection can favour large female size (i.e. Erlandsson & Johannesson 1994; Emlen & Wrege 2004), and no study to date has shown sexual selection favouring small female size (reviewed by Blanckenhorn 2005). This, I believe constitutes one of the limitations of the study of SSD.

Second, phylogenetic comparative methods are useful, but limited tools of research. Comparative methods such as independent contrasts (Felsenstein 1985) and generalised least squares (Pagel 1997, 1999; Garland & Ives 2000; Freckleton et al. 2002) are excellent tools for revealing macro evolutionary patterns; nevertheless, these methods

are correlational. Therefore, these methods do not separate cause from effect, making it difficult to draw any conclusions on the origin of the diversity of SSD exhibited by a given taxa. One other method, Discrete (Pagel 1994) has been designed to detect correlated evolution and the order of evolution between pairs of traits. Nevertheless such method is still correlational, since it asks whether the data and phylogeny fit one model of evolution better than another, which lends support to the most likely order trait evolution (Thomas, G. H. pers. comm.). Furthermore, the trends revealed by phylogenetic comparative methods should be, when possible, assessed empirically in order to broaden our understanding of the processes behind the patterns of SSD.

Third, there is a lack of studies investigating the possible causes of Rensch's rule. Whilst several hypotheses have been proposed to explain this macroecological allometric pattern (Fairbairn 1997), such hypotheses have rarely been tested (but see Székely et al. 2004; *Chapter 5*).

Finally, there is a prevalent, simplistic view that large size is always better (Thompson & Fincke 2002). Thompson & Fincke (2002) criticise this view by arguing that if large size provided fitness benefits to all taxa, animal lineages would show the tendency to increase in size over time. Several studies have now demonstrated that large size has reproductive advantages in certain taxa (Endler 1986; Andersson 1994; Blanckenhorn 2000; Kingsolver et al. 2001; Serrano-Meneses et al. in press), but also, it has been shown that small male size can also be favoured by sexual selection (see Blanckenhorn 2005). The development of large size and adult size itself is expected to be penalised or regulated by natural selection (Blanckenhorn 2000); however, selection for large body size predominates in nature (Kingsolver & Pfenning 2004), leading to phyletic size increase over time (Cope's rule; Cope 1896; Kingsolver & Pfenning 2004) even when this increase in size accelerates the rates of extinction (Martin 1984; LaBarbera 1986; Arnold et al. 1995). There are of course, exceptions to this rule (Kingsolver & Pfenning 2004); in such taxa, selection for decreased developmental time may halt the selection for increased size, whereas in taxa that exhibit Cope's rule, selection for increased size may predominate over selection for decreased developmental time (Kingsolver &

Pfenning 2004). More research is needed to determine whether overall selection on large size versus selection on developmental time is variable across taxa and whether this variation is likely to influence Cope's rule (Kingsolver & Pfenning 2004).

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Chapter 2. *Sexual size dimorphism in the American Rubyspot: male body size predicts male competition and mating success*

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Abstract

Sexual differences in body size are widespread amongst animals, and various explanations for the evolution and maintenance of sexual size dimorphism (SSD) have been proposed. Here we investigate the effects of sexual selection and fecundity selection on the sizes of males and females, respectively, in American Rubyspots (*Hetaerina americana*). This damselfly is sexually dimorphic; males are larger than females, and the males sport large red spots at the base of each wing that are sexually selected *via* male-male contests. Males defend territories along streams and rivers, and mating success is determined by the ownership of a territory. Firstly, we show that large males hold territories for longer and sustain longer territorial fights than small males. Territorial males obtain more copulations than non-territorial ones. Large males also exhibit higher proportions of wing pigmentation, and they mate with large females. Secondly, large territorial males have high energy reserves, whereas non–territorial males appear to have depleted reserves. Thirdly, selection analyses of body size show disruptive selection acting on male body size, suggesting that both small and large males may be favoured in terms of mating success. We also test whether fecundity selection is acting on female size. However, female body size was unrelated to the number of eggs the female carried. Taken together, our results suggest that in this territorial damselfly species male-biased size dimorphism is driven by large male size in male-male competition being selectively advantageous in territory acquisition and/or maintenance. We also suggest that small size is advantageous in no-territorial males to improve their agility in courting (or subduing) females.

1. INTRODUCTION

Differences in body size between males and females are widespread in the animal kingdom. The degree and direction of body size difference, termed sexual size dimorphism (SSD), varies across different animal taxa (Andersson 1994; Teder & Tammaru 2005). Several hypotheses have been advanced to explain the interspecific variation in SSD (reviewed by Shine 1989; Andersson 1994; Blanckenhorn 2005). First, increased female body size relative to male size (female-biased SSD) may be the result of selection for fecundity (Andersson 1994). This is likely to happen, if large females have higher reproductive success due to their higher capacity for producing eggs (Ridley & Thompson 1979; Wiewandt 1982; Honěk 1993), and/or if large females are preferred by males (Sandercock 1998, 2001). For instance, a positive relationship between female size and fecundity has been found in frogs, spiders and insects (Shine 1979; Head 1995; Prenter et al. 1999; Legaspi & Legaspi 2005).

Second, differential exploitation of resources may reduce the competition between the sexes, and drive their sizes to different optima (differential niche–utilisation, Selander 1966; Hedrick & Temeles 1989; Shine 1989; Thom et al. 2004). If resources are scarce and a differential exploitation between the sexes arises, then changes in morphology and body size may follow (Shine 1989; Sandercock 2001; Temeles & Kress 2003).

Third, sexual selection acting on either sex may select for SSD (Raihani et al. 2006). For instance, male-male competition may favour large body size in those species in which males compete intensely for females (Mitani et al. 1996; Dunn et al. 2001; Lindenfors et al. 2003; Raihani et al. 2006). Thus, large size may be advantageous for males in polygynous species (Clutton-Brock & Harvey 1977; Owens & Hartley 1998).

Finally, the selective advantage of body size may depend on whether the competition occurs on the ground or in the air (Payne 1984; Jehl & Murray 1986). If males compete or display in the air then small male size may be advantageous (Andersson & Norberg 1981; Blomqvist et al. 1997; Székely et al. 2000, 2004; Serrano-Meneses & Székely

2006), whereas large size may be beneficial in those species where males display or compete on the ground (Clutton-Brock et al. 1982; Anderson & Fedak 1985; Lindenfors & Tullberg 1998). These selective processes may be reinforced *via* female choice (reviewed by Thornhill & Alcock 1983; Choe & Crespi 1997).

The effect of sexual selection on SSD may vary across insect taxa. It is possible that different mating strategies (i.e., territoriality, non–territoriality) promote changes in the degree and direction of SSD (Andersson 1994). For example, small size is often advantageous to non-territorial individuals in situations where agility is important (Fincke 1988; Neems et al. 1990), whereas large size is often linked to territorial advantages (Alcock 1979; Fincke 1984; Tsubaki & Ono 1987; Crespi 1988; Villalobos & Shelly 1991; Polak 1993), presumably due to the fat-storing ability of large males to fuel aerial fights (Marden & Waage 1990; Plaistow & Siva-Jothy 1996; Plaistow & Tsubaki 2000; Contreras-Garduño et al. 2006).

Odonates (dragonflies and damselflies) are an ideal group for investigating the selective and physiological processes underlying SSD. They exhibit a variety of mating tactics, strategies and habitats (i.e. Forsyth & Montgomerie 1987; Plaistow & Tsubaki 2000), causing different selective pressures on body sizes of males and females (Conrad & Pritchard 1992; Thompson & Fincke 2002); their SSD ranges from female-biased to male-biased SSD (Anholt et al. 1991). Females are usually the larger sex in adult odonates (Anholt et al. 1991), but this difference can be less evident, or even the opposite, in territorial species (Anholt et al. 1991; Fincke et al. 1997). What selective processes influence male- or female-biased SSD? On the one hand, it is possible that male body size is under selection toward large size in species with territorial mating systems (Anholt et al. 1991) since male-male competition is known to select for large male body size (relative to female size, Blanckenhorn 2005). However, the relative strength of different selective processes may be difficult to detect. For example, stabilising selection may also act on body size across odonates (reviewed by Thompson & Fincke 2002). When stabilising selection acts, fitness is not a linear function of size. For instance, males of intermediate size of the non-territorial damselfly *Enallagma*

hageni exhibit higher lifetime reproductive success than large males (Fincke 1982). Furthermore, disruptive selection may also occur if, for example, both large and small males are favoured. The conditions of this selection are variable although it often accompanies assortative mating (e. g. Jones et al. 2003). On the other hand, selection for increased female fecundity may result in selection for increased female body size relative to male body size, if fecundity increases with body size. Nevertheless, the relationship between female fecundity and body size is poorly understood in odonates (Cobet 1999).

In territorial odonates males fight for the acquisition of a territory (Corbet 1999). Having a territory is often a pre–requisite for males to obtain copulations, because females are attracted to these areas for copulation and/or oviposition (Corbet 1999; Córdoba-Aguilar & Cordero-Rivera 2005). Fights over a territory may be short (i.e. 3 – 5 sec), or long (from 20 minutes to over 2 hours), however, territory acquisition is usually determined by prolonged encounters (reviewed by Córdoba-Aguilar & Cordero-Rivera 2005). Particularly, in Calopterygidae the acquisition of a territory is usually determined by the outcome of aerial encounters between territory holders and intruders (Córdoba-Aguilar & Cordero-Rivera 2005). In these prolonged encounters, males with higher energy reserves in the thoracic muscles (metabolic fat) have an advantage over males with low fat reserves (Marden & Waage 1990; Plaistow & Siva–Jothy 1996; Koskimäki et al. 2004; Contreras-Garduño et al. 2006).

Here we investigate a damselfly, the American Rubyspot *Hetaerina americana,* to reveal whether male-biased SSD is driven by sexual selection operating on male body size. First, we predict that large body size is advantageous for males. The American Rubyspot exhibits a resource-defence polygyny so that males compete intensely over the possession of a territory (Grether 1996a, b). Soon after emergence, males develop a large red-pigmented spot at the base of each wing. Grether (1996a, b) provided experimental evidence showing that the red pigmentation is involved in male-male interactions, since males with larger wing spots held territories for a greater proportion of their reproductive lifespan and, therefore, mated at higher rates. Grether (1996b) also

found that body size was positively selected, but only in non-territorial males, possibly because they were able to subdue females in the pre-copulatory stage. Nevertheless, the relationships between body size, wing pigmentation and male mating success have not been investigated. In this study we explore the interactions of these variables and the role of fat reserves and muscle mass in the context of the advantage of large body size in territoriality. We also use selection analyses to quantify the direction and mode of selection acting on male body size in relation to their mating patterns. Second, fecundity selection has not been investigated in American Rubyspots, thus we also investigate whether the number and size of the eggs relate to female size. These relationships may be linear suggesting directional selection for increased female body size. Alternatively, females of intermediate body sizes may more fecund than large or small females. The latter scenario would be consistent with stabilising selection.

2. MATERIALS AND METHODS

(a) Study site

Fieldwork was carried out in Tehuixtla, Morelos, Mexico (18° 32' 56" N, 99° 16' 23" W, elevation 840 m) between 17 December 2003 and 27 February 2004, and between 12 November and 15 December, 2004. We worked along the shore of the Amacuzac River in a section approximately 300 m long. Since American Rubyspots avoid areas with shade or cover (M. A. S.-M. personal. observation), we divided our study site to three areas that were not shaded by trees and held the largest concentrations of individuals.

(b) Marking, morphometrics and body size

On each day unmarked animals were caught and marked with an indelible marker on the right anterior wing using a unique combination of three digits. These numbers were easily readable through binoculars from a few metres, and allowed us to identify individuals during behavioural observations and daily surveys. First, for each captured male we measured their body length (from head to the tip of abdomen), head width, wing length (right anterior wing) using a digital caliper (to the nearest 0.01 mm).

Second, the wings of 211 randomly chosen marked males were photographed at constant distance using a digital camera (Olympus 765UZ). Third, males were categorised as either territorial or non-territorial. In our study site territorial males defended an area against conspecifics and remained faithful to their area after an aggressive dispute, whereas non–territorial males did not establish an area, wandered along several sections of the river and were chased off by territory owners (for a review on the territorial behaviour see Corbet 1999). Finally, male age was assigned to three age classes: (i) juvenile mature, (ii) mature and (iii) old (see Córdoba-Aguilar, 1994 for a detailed description of this procedure). These age classes were estimated from morphological cues (Córdoba–Aguilar 1994): (i) juvenile matures exhibit bright intense colours and their wings are highly transparent; (ii) mature individuals show less brightness and intensity in body colour, their wings are less transparent than that of the juvenile mature individuals; (iii) old individuals usually show dark body colouration and their wings tend to be broken at the tips.

We use body length as a *proxy* for body size for two reasons. First, wing length and head width are highly correlated with body length (see Results). Second, we argue that measuring SSD from differences in wing length may not be appropriate, since it leads to the false conclusion that females are larger that males in this species (see Results).
In this work we assume that body size is fixed after adult emergence, although one may argue that male body length may change so that it reflects feeding condition. To test this assumption we captured 44 adult males in our study site on 27th of May 2006, measured their total body length, and allocated them alive into individual plastic tubes. The tubes were transported to a laboratory at UNAM inside a plastic cooler to keep males alive but inactive. In the laboratory we choose 22 individuals randomly, and fed these males whereas the remaining 22 males were starved. Males in the fed group were manually fed using frutiflies *Drosophila melanogaster* until they ate no more flies (usually, they took 6 - 11 flies before saturation). Males were fed once a day for 2 days. After 48 hours of capture their body length was re-measured.

(c) Male behaviour, territorial tenure and survival

Male behaviour was recorded daily between 10:00 and 15:00 hours (Central Standard Time) when American Rubyspots are most active (M.A. S.–M. pers. obs.). Each male was observed for 15 minutes by scan sampling. Behavioural units were recorded every 10 second using a digital timer. We then estimated the proportion of time the males spent fighting. Prior to analyses, these proportions were log_{10} transformed, and we refer to these data as 'fighting rate'.

During behavioural observations and daily surveys we also recorded copulations, and noted whether the observed male was territorial or not. We use the number of copulations as an indicator of male mating success.

To estimate male territory tenure (the number of days a male held a territory), and survival (the number of days a male was seen alive) we searched for marked animals from 12:00 to 14:00 hrs. Both territorial tenure and survival were log_{10} transformed. Survival analyses only included those males that were marked during the first 15 days of the first field season (December 2003 – February 2004), to exclude those individuals whose lifetime was not fully covered by the study time span.

(d) Wing pigmentation

Pigmented patches and total wing areas were measured on digital photographs of 211 males using ImageJ 1.34s (National Institutes of Health, http://rsb.info.nih.gov/ij/). We measured the total area (in pixels) of the four wings and the area of their respective pigmented patches. Since we were interested in the relationship between wing pigmentation and at a given body size, we controlled in two ways for the allometric relationship that large wings bear large pigmented patches. First, we estimated the average proportion of wing pigmentation for four wings (see similar approach by Córdoba-Aguilar et al. 2003). Second, we calculated the log_{10} (mean areas of the pigmented patches) and the log_{10} (mean wing areas), and used these log-transformed values in the analyses (see below).

(e) Fat extractions and flight muscle mass

Weights of metabolic fat and flight muscle were measured for 22 territorial and 22 non–territorial males that were captured in the field. We used the thorax of males to measure both metabolic fat and flight muscle since they are mostly found in this cavity (Plaistow & Siva–Jothy 1996; Corbet 1999). Fat extractions were based on the method described by Marden (1989), where available fat is measured as the difference between thorax dry weight and thorax weight after fat extraction by petroleum ether (see Marden 1989 for full details); therefore, fat load refers to fat weight in grams. The dry, fatless thorax was later immersed in 0.2 M potassium hydroxide for 24 hours (Plaistow & Siva–Jothy 1996). After this treatment, flight muscle was digested and the remaining cuticle was washed in distilled water, dried and re–weighed. The difference between the weight of thorax cuticle with muscle and the dry muscle-less cuticle was used to estimate muscle mass in grams.

(f) Fecundity

45 females were captured in the field, and stored in 70% ethanol until dissected in the laboratory. We also measured their head width, body length and wing length to the nearest 0.01 mm. We only captured those females that attempted oviposition following copulation. These females have presumably matured a batch of eggs that were ready to be laid. In the laboratory, each female was placed in a Petri dish filled with water for two hours and dissected under a stereo-microscope by removing the abdominal sternites and gut. Since clutch size is often traded off against egg size (Roff 2002), we also measured the length and width of 10 eggs per female using a 10x measuring eyepiece, and used the average size of these eggs in the analyses. Egg size was estimated by calculating an egg-index based on egg-length x width^2.

(g) Statistical analyses

We compared the body size of males and females using independent samples *t*-tests. To test whether body length changes with feeding regime and thus with body condition, we used paired-samples *t*-test to compare the body length between prior and after treatment in both fed and starved groups. We investigated the relationships between male size (ie

body length or wing length) and fighting rate, territory tenure or survival using Pearson's correlations. We constructed two General Linear Models (GLMs) that initially included either body length or wing length as dependent variable, and male fighting rate, territory tenure, survival as explanatory variables, and then removed the non-significant variable(s) using backward elimination. All first-order interactions were tested in the initial models, but none was significant ($p >> 0.3$), thus statistical interactions were not considered further.

To test the relationship between wing pigmentation and body size, we first investigated whether the proportion of wing pigmentation was related to body length in a Pearson's correlation. Second, we fitted a major axis regression (MA; model II regression, Sokal & Rohlf 1981) between log_{10} area of pigmented patches (dependent variable) and log_{10} areas of wing, We used major axis regression instead of least squares regression since the latter does not take into account that both X and Y are estimated with error, and the magnitude of errors were likely different between X and Y variables (Sokal & Rohlf 1981). A MA slope significantly greater than one would suggest that large males have a higher proportion of wing pigmentation. The MA slope and its 99% confidence intervals (lower CI – upper CI) are provided. The confidence intervals of the slope were calculated by bootstrapping the log_{10} transformed data using R (R Development Core Team, http://www.R-project.org).

We investigated the relationships between either fat load or muscle mass (as dependent variable), and body length (independent variable) by considering the possession of a territory (ie male status) using two GLMs. In Model 1 fat load was the dependent variable, body length was a covariate, and male status was a factor. We found a significant interaction between male status and body length ($p = 0.001$). Thus, we investigated further the association between fat load and body length separately for territorial and non–territorial males using bivariate least squares regressions. In Model 2 muscle mass was the dependent variable, body length was a covariate and male status was a factor. Male status * body length was not significant ($p = 0.488$), therefore this interaction was not included in Model 2.

Assortative mating in regard to body size was tested by fitting a MA using the body lengths of 54 males and females found in copula. We use MA for two reasons. First, body size is usually estimated with error so that least squares regressions may not be appropriate. Second, least squares regression often underestimates the slope and the confidence intervals when both variables are measured with error (Fairbairn 1997). The slope of MA regression and its 99% confidence intervals (lower CI – upper CI) are provided. Confidence intervals were calculated by bootstrapping the body length data using R (R Development Core Team, http://www.R-project.org).

We tested the relationship between female body length and egg number using Pearson's correlation. Unexpectedly, egg size was not normally distributed (skewed toward left, Kolmogorov-Smirnov $Z = 2.26$, $n = 45$, $p = 0.001$), thus we used Spearman's rank correlation for the relationship between female body length and egg size. Data are shown as mean ± STD and the analyses were carried out using SPSS Ver. 12 with the exception of preceding statements.

(h) Selection analyses

In selection analyses we use two measures of body size: body length and wing length. Prior to the selection analyses we performed a principal component analysis (PCA) to reduce the number of variables. Nevertheless, the eigenvectors of both body length and wing length were high (0.707, 0.707; respectively) suggesting that body length and wing length were similarly represented in the PCA. Wing pigmentation was not included in the selection analysis due to low sample size (data were available only for 16 mated males).

We estimated the direction and mode of selection acting on body length, wing length and male age using a multiple regression analysis (Lande & Arnold 1983). To estimate directional (β_i coefficients) selection and curvilinear (stabilizing/disruptive and correlational: γ_{ij} coefficients) selection, we used partial linear regression and quadratic multivariate regression respectively, of relative fitness against standardised body length, wing length and age (mean = 0, $S^2 = 1$) as independent variables. Relative fitness (w_i) of

a given male was estimated as $w_i = W_i/\hat{W}$, where W_i is the number of matings obtained by a male i throughout the whole season and $\hat{W}$ is the mean number of matings of all males in the population.

(i) Ethical note

All animals were treated as humanely as possible and released immediately after marking and measuring. The total handling time of every individual was usually less than three minutes. Marking was apparently not harmful since shortly after release most territorial males returned to their territory, whereas non-territorial males and females returned to perching sites near the river. No permission was required by SEMARNAT (Mexico) to carry out this work.

3. RESULTS

(a) Sexual size dimorphism

Males were significantly larger than females as measured by body length ($t_{844} = 35.04$, $p = 0.001$), head width ($t_{844} = 9.65$, $p = 0.001$) and body mass ($t_{23} = 2.15$, $p = 0.042$). Females, however, had longer wings than males ($t_{844} = 4.58$, $p = 0.001$, Figure 1). Wing length and head width are highly correlated with body length (wing length: males, $r_{738} = 0.76$, $p = 0.001$; females, $r_{104} = 0.71$, $p = 0.001$; head width: males, $r_{738} = 0.84$, $p = 0.001$; females, $r_{104} = 0.78$, $p = 0.001$).

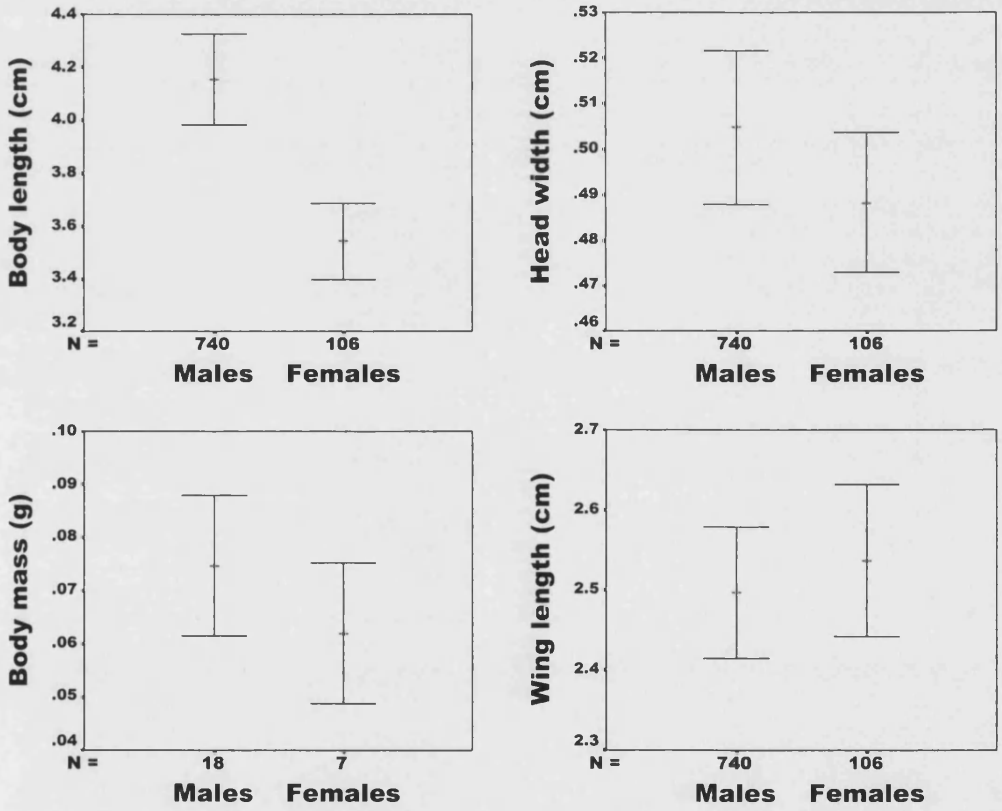

Figure 1. Body sizes of male and female American Rubyspots using different morphological characters (mean ± STD). N refers to the number of males or females.

Male body length did not change in either feeding regime; male length was not different before and after treatment either in the fed group ($t_{21} = 0.48$, $p = 0.634$) or in the starved group ($t_{21} = 1.04$, $p = 0.309$).

(b) Territoriality and survival

Although territorial males were not significantly larger than non-territorial males ($t_{384} = 1.33$, $p = 0.183$), both territory tenure and male fighting rate increased with body length ($r_{83} = 0.34$, $p = 0.002$, Figure 2a; $r_{54} = 0.44$, $p = 0.001$, Figure 2b). These relationships were weaker with wing length (territory tenure: $r_{83} = 0.15$, $p = 0.175$; fighting rate: $r_{54} = 0.26$, $p = 0.056$). Territory tenure was unrelated to the age of males (age estimated at capture, one-way ANOVA: $F_{2,84} = 2.46$, $p = 0.092$), and it was invariable across areas within the study site ($F_{2,84} = 0.39$, $p = 0.678$).

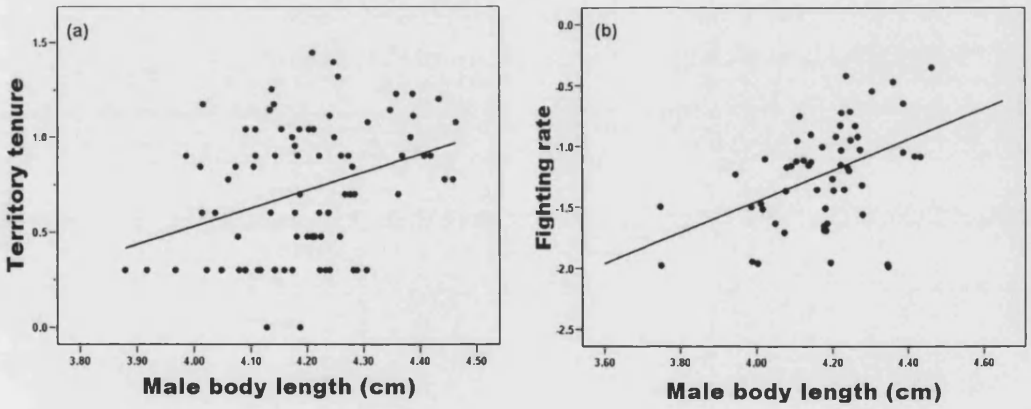

Figure 2. Body length of territorial males in relation to (a) territory tenure, and (b) fighting rate.

Body size was unrelated to survival (body length and survival: $r_{211} = 0.07$, $p = 0.316$; wing length and survival: $r_{211} = 0.07$, $p = 0.281$). These results were consistent with the GLM of body length, since survival was not retained in the final model, whereas both territory tenure and male fighting rate were correlated with male body length ($F_{1,33} = 4.64$, $p = 0.039$; $F_{1,33} = 11.33$, $p = 0.002$, respectively). None of the explanatory variables were significantly related to wing length ($p > 0.155$ in all cases).

(c) Male wing pigmentation and body size

The proportion of wing pigmentation was 0.130 ± 0.021 (n = 211 males). Large males had a higher proportion of wing pigmentation than small males as measured by body length (r_{209} = 0.29, p = 0.001; Figure 3) or wing length (r_{209} = 0.25, p = 0.008).

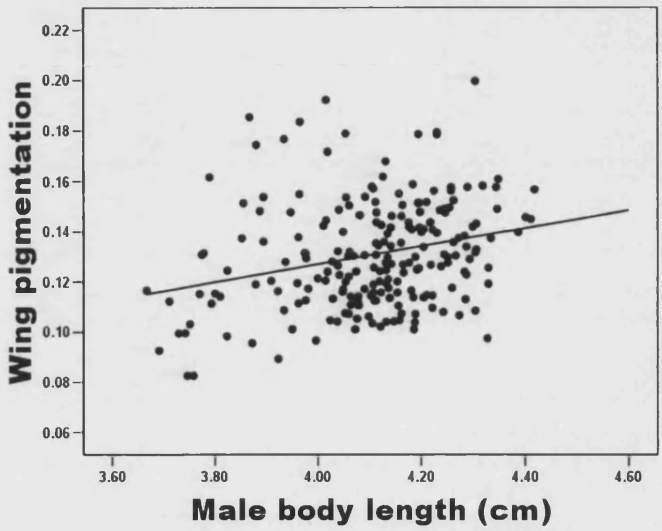

Figure 3. Wing pigmentation increases with male size (r_{209} = 0.29, p = 0.001). Note that wing pigmentation is expressed as proportion, i.e. pigment area * wing area^{-1}.

The log_{10} (area of pigmented patches) and log_{10} (area of wings) are highly correlated (b = 1.31, n = 211). The slope of major axis regression is significantly greater than one (lower 99% CI – upper 99% CI: 1.25 – 1.37).

(d) Fat load and flight muscle mass in relation to body size

Fat load related differently to body length in territorial and non-territorial males (Model 1, male status * body length p = 0.001; Figure 4a): fat load increased with body length in territorial males (r_{20} = 0.69, p = 0.001), whereas it was unrelated to body length in non–territorial males (r_{20} = 0.27, p = 0.215).

Flight muscle mass increased with body length (Model 2: $F_{1,41} = 59.89$, $p = 0.001$), and it was not different between territorial and non–territorial males ($F_{1,41} = 0.50$, $p = 0.484$, Figure 4b).

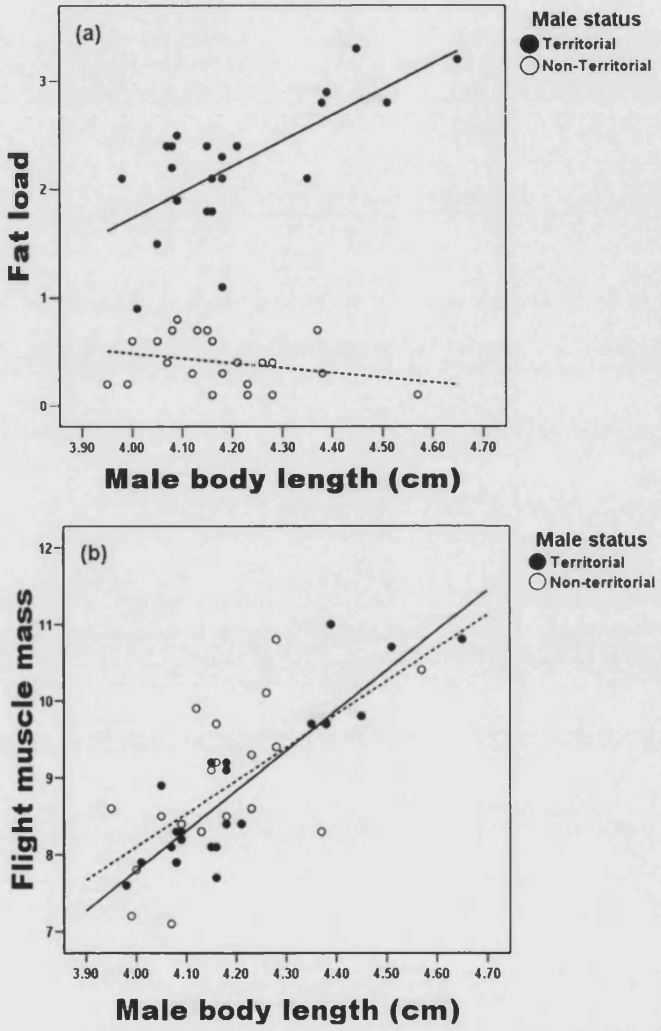

Figure 4. Body length in relation to (a) fat load (g x 10^{-3}), and (b) muscle mass (g x $10^{-3)}$ in territorial and non–territorial males.

(e) Mating rate, selection analysis and assortative mating

Males mated 0.014 ± 0.006 times per day. The maximum number of observed copulations for a single male was 3. Out of 206 territorial males, 49 were seen in copula,

whereas only 13 out of 180 non-territorial males were seen copulating. Thus territorial males obtained more copulations than non-territorial males ($\chi^2 = 19.55, p = 0.001$).

We found directional selection on male body length, but not on wing length or age (Table 1a). The positive and significant gradient of selection on male body length (β_i) suggests that larger males have higher mating success. The gradients of non-linear selection (γ_{ii}) were not significant except for male body length (Table 1a). This gradient of selection was positive indicating that disruptive selection is acting on male body length. Thus, both large and small body size are being selected for. No correlational selection was significant.

Table 1. (a) Directional (β_i), quadratic (γ_{ii}) and correlational (γ_{ij}) selection gradients for body size and age on mating success in territorial and non-territorial males. (b) ANOVA for the models. Standard errors are indicated in parentheses. * p = 0.006; ** p = 0.028; ns, not significant.

a. Character	β_i	γ_{ii}	γ_{ij}
Body length	0.519(0.189)*	0.393(0.178)**	
Wing length	0.132(0.189) ns	0.016(0.153) ns	
Age	-0.007(0.125) ns	-0.080(0.104) ns	
Body length x Wing length			-0.123(0.272) ns
Body length x Age			0.182(0.207) ns
Wing length x Age			0.019(0.197) ns

b. ANOVA for the linear model

Source	df	SS	F	p	Adj. R^2
Model	3	290.250	8.437	0.001	0.029
Error	737	8451.875			

ANOVA for the quadratic model

Source	df	SS	F	p	Adj. R^2
Model	9	486.403	4.785	0.001	0.044
Error	731	8255.721			

The body lengths of males and females found in copula were highly correlated (Figure 5; b = 0.77, n = 54 pairs). The slope of major axis regression was not different from one (lower 99% CI – upper 99% CI: 0.47 – 1.19).

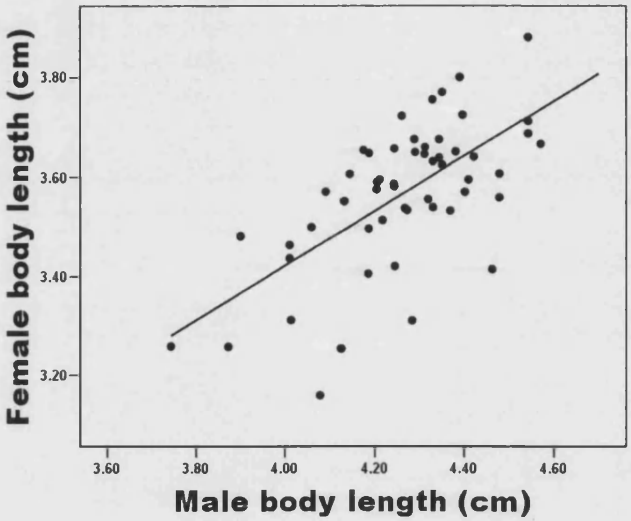

Figure 5. The relationship between male body size and female body size found in copula. The continuous line represents the fitted relationship using major axis regression (b = 0.77, n = 54 pairs).

(f) Female fecundity

Females had 676.94 ± 118 eggs (n = 45 females), and their egg size was 0.048 ± 0.001 mm^3 (n = 45 females). Female body length was not related either to the number (r_{43} = 0.04, p = 0.774), or the size of her eggs (Spearman's rank correlation r_s = – 0.04, n = 45, p = 0.752).

4. DISCUSSION

Our study suggests that body size does not reflect feeding condition in adult male American Rubyspots. We also found that large body size is selectively advantageous in this territorial damselfly. In the American Rubyspot males are larger than females; this, however, depends on the trait we measure. For instance, females have longer wings than

males but this should not be interpreted as females are overall larger than males, since all other measures produced the opposite direction of SSD. Thus ecological processes appear to influence differently wing length from other parts of the body. For instance, Taylor & Merriam (1995) found that long-winged males and females *Calopteryx maculata* were more likely to occur in open habitats, such as grasslands, than in forests. Taylor & Merriam (1995) argued that large wings would reduce manoeuvrability in dense habitats such as forests. Another reason for the wing morphological difference may be the type of flights performed by males and females. For instance, it is suggested that females perform more prolonged flights whereas males perform forward flights. This is because long (and wider) wings seem to be used for prolonged flights (Marden 1987) rather than for forward flight (Wootton 1992).

The body lengths of territorial and non-territorial males were not different; nevertheless, larger males have a greater proportion of fat reserves and flight muscle than smaller males. The latter result may be expected in territorial odonates. Males start building up fat reserves soon after emergence, and these reserves are burnt during sexual activities and territorial disputes (Plaistow & Siva-Jothy 1996). Perhaps large male size at emergence provides the opportunity to produce large muscular mass and store more fat in the days following eclosion (Plaistow & Siva-Jothy 1999). Once a male has lost his territory, there is a large decrease in fat storage (Marden & Waage 1990; Plaistow & Siva-Jothy 1996). Low fat reserves (although not as low as in the evicted territorial males) are seen in non-territorial males that have never defended a territory (Contreras-Garduño et al. 2006). This pattern has been shown in other *Calopteryx* species (Marden & Waage 1990; Plaistow & Siva-Jothy 1996) as well as in *Hetaerina americana* (Contreras-Garduño et al. 2006). The decrease after energy-demanding contests, despite large male size, is therefore inevitable. Thus after territory eviction large size is no longer an indicator of fat reserve storage although it was previously. Note that in American Rubyspots non-territoriality includes both males that were once territorial and males that tried to obtain a territory but failed to do so. Current work is looking at whether these two types of non-territorial animals differ in size.

Large body size is advantageous for male rubyspots since it enhances territory tenure, fighting rate, wing pigmentation and mating. Large males held territories for longer possibly due to higher fighting potential ability (i.e. large males had higher fighting rates, perhaps as result of having large fat stores in their flight muscle). Note that territory tenure was unrelated to the age of males, suggesting that the ability of males to remain territorial did not depend on age. Interestingly, Grether (1996 b) found that males with large wing pigmentation held territories for a higher proportion of their reproductive life in both an observational and an experimental study in which the wing spot of a number of males was artificially enlarged. However, and unlike our results, Grether (1996 b) reported that body size was unrelated to territory tenure. We argue that these discrepancies arise from (i) the differences in estimating body size between Grether (1996 b) and our study, and (ii) from ignoring the relationship between wing pigmentation and body size. Whilst Grether (1996 b) estimated body size from wing length, wing width and thorax width, we used body length. Perhaps large size allows larger muscle mass and more fat (Plaistow & Siva-Jothy 1999) that may be honestly signalled to other rivals by using wing pigmentation during territorial contests (since large males are more ornamented). Grether (1996 a, b) argued that wing pigmentation has evolved via male-male interactions, as it is displayed by males during agonistic encounters. In territorial males fat reserves are positively correlated with wing pigmentation, whereas the same is not true for non-territorial males (Contreras et al. 2006). The fact that fat reserves are related to wing pigmentation in territorial males strengthens the idea that wing pigmentation is an honest signal of body condition only in territorial males. However, to assess the relative significances of these processes one needs to carefully evaluate the costs and benefits of pigmentation in both female-choice and male-male competition.

Male survival was unrelated to body size. We suggest three explanations for this. One explanation is that more ornamented individuals (i.e. with large wing spots) may be spotted sooner by their preys, so that they face reduced food intake and thus survival (Grether & Grey 1996): in an experiment females that were marked with red spots in the wings (like those of males), Grether & Grey (1996) found that experimental females

captured less prey compared to control females, thereby gaining less weight. Thus, according to Grether & Grey (1996) wing pigmentation enhances the prey's ability to detect and escape from the approaching damselfly. This may apply particularly to large damselfly males in which strong selection may operate against large spots. A second explanation is that large territorial males may fight until exhaustion and be more likely to die than a small animal, which makes survival estimates difficult to be correlated with size. Our study supports this notion since large males spent more time on fighting. Finally, our results may also be due to the way we estimated survival so that some males may be missed due to dispersal and not mortality. Ideally, survival should be estimated from mark–recapture data, taking into account differences in resighting rates (reviewed by Lebreton et al. 1992). In American Rubyspots, however, survival is difficult to measure accurately given their strong flying capacity.

Territoriality was related to mating success since territorial males obtained more copulations than non-territorial males. One may argue, however, that we underestimated mating success, particularly of non-territorial males by failing to observe copulations. However, in our study both territorial and non-territorial males were highly visible suggesting that our estimates of copulation rates are not biased (both can be seen crossing the river, looking for oviposition sites as females do not lay eggs in the male's defended space; M. A. S.-M. unpub. data). Furthermore, non-territorial males obtained few copulations in other studies too in *Hetaerina* species (e. g. Córdoba-Aguilar 1995; Grether 1996 a). Females visit the territories once they are ready to mate, and although precopulatory events have not been described in detail in this species (but see Johnson 1963), it seems that matings are more likely to occur with territorial males as occurs in other calopterygids (Plaistow & Siva-Jothy 1996; Córdoba-Aguilar & Cordero-Rivera 2005). One explanation for the differential mating success of territorial *versus* non-territorial males is that only a territorial male may grant good oviposition sites to females (Alcock 1987; Waage 1987; Meek & Herman 1990), and provide protection from other males' interference (Siva-Jothy 1999). In *Hetaerina*, unlike *Calopteryx*, the couple goes to a place distinct to that defended by the mating male to lay eggs (Córdoba-Aguilar & Cordero-Rivera 2005). In fact, female visitation rate is not affected by

oviposition sites (Alcock 1987). In this situation, the couple faces aggressive attacks by other males when crossing over those animals' territories (M. A. S-M unpub. data). Possibly during these instances, a territorial male may firmly hold the female and prove his flying ability to provide the oviposition resource (Córdoba-Aguilar & Cordero-Rivera 2005). This holding capacity may not be provided by a non-territorial male.

Our selection analysis corroborated our previous findings that large size is selectively advantageous in males. However, it also detected disruptive selection (both extremes of male body length are selected in mating success) that is unusual in odonates. Among odonates directional selection on body size is expected in resource defense polygamist species such as *H. americana* (Fincke et al. 1997), although the males only defend a place but once the couple is formed, they look for an oviposition site. Although stabilising selection has been found in non-territorial species (i.e. Fincke 1988), it is also in these species where selection for small males has been found (i.e. Banks & Thompson 1985; Anholt 1991) presumably because of advantages *via* better flight manoeuvrability (Fincke et al. 1999). This can be the case of *H. americana* in which this can be advantageous during territorial fighting and chasing or while evading other males when the animal is in tandem and looking for a place for the female to lay eggs. Theoretical studies suggested that disruptive selection is expected to be accompanied with assortative mating (i.e. Bürger & Schneider 2006), and this prediction is consistent with our results. There may be two explanations for this pattern. First, large and successful males may prefer to mate with large females, perhaps because these are more fecund. Note, however, that our results do not support the relationship between female body size and fecundity. It is therefore not possible to draw any conclusions on whether linear or stabilising selection act on female body size. Perhaps a better estimate of female fecundity would be lifetime female fecundity. Studies of lifetime female fecundity (see Corbet 1999) reported associations between female body size and lifetime fecundity in *Ischnura graellsii* (Cordero 1991) and *Coenagrion puella* (Banks & Thompson 1987). However, contrary to the hypothesis that female fecundity increases with body size, the optimum female size for lifetime egg production revealed that females of intermediate size produced the most eggs (Banks & Thompson 1987). Although more studies are

needed using lifetime female fecundity, assessing these are challenging given that many calopterygids do not survive in the laboratory. Second, assortative mating may emerge from a simple mechanical rule, for instance, if only males and females of similar sizes can copulate (mechanical barrier, Arnqvist et al. 1996). An effective fit of the structures involved in tandem connection (male abdominal appendages and female mesostigmal plates) would be advantageous again during those flights that the couple takes to reach an oviposition place. A third explanation would be related to the reduced energetic advantage and better manoeuvrability that a small male can carry when flying in couple with a small female than with a large female. Reasons one and three however would not explain why small males should pair with large females and large males with small females respectively. The second possibility, tandem fitting, is currently under investigation.

In conclusion, sexual selection is likely to select for large size in male-male competition in American Rubyspots, since large males hold territories for longer than small males and can store more fat. The fighting ability is likely to be signalled by the wing spot. However, when it comes to mating success, disruptive selection on male body size was documented although the reasons for this remain to be clarified. The advantage of large size is less apparent in females, since we found no relationship between female size and fecundity. Further research should estimate fecundity from the number of eggs deposited in all oviposition events. To quantify lifetime selection on male and female body size, future studies should use quantitative genetic protocols using selection differentials (Preziosi & Fairbairn 2000; Blanckenhorn 2005).

Odonates exhibit an excellent range of mating strategies and ecological traits (Corbet 1999). Thus future works should test functional hypotheses of SSD (i.e. fecundity selection, niche-division, and sexual selection) using phylogenetic comparative methods. Recent advances in phylogenetic methods now allow to test whether evolutionary changes towards territorial mating systems are related to changes towards male-biased SSD. Furthermore, it can be tested whether male body size has changed around female body size as to produce the observed patterns of SSD or *vice-versa.*

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Chapter 3. *The effect of larval diet on adult sexual differences in size and fat reserves in a tropical territorial damselfly*

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Manuscript

Details and extent of the contributions by authors

M. A. Serrano-Meneses: data collection, statistical analyses, manuscript writing (60%)

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Abstract

Sexual size dimorphism (SSD), a difference in size between males and females, is a prevalent morphological trait exhibited by many animal species. Adult body size is usually affected by a number of environmental factors (such as available feeding resources) during development. Therefore it is likely that the early stages of development are a critical period for animals to attain resources that will influence their reproductive success. Here we investigate how food resources during the larval stage of the territorial American Rubyspot damselfly (*Hetaerina americana*) may influence adult body size and fat reserves. Larvae were collected from their natural environments and reared in one of three diet regimes: (i) rich, (ii) intermediate and (iii) poor. Food regimes, however, did not influence adult body size or fat reserves of either males or females. We discuss four potential explanations for these results. First, food alone may not be the sole determinant of adult body size, but the interplay between genetic and other environmental variables. Second, the effect of diet regimes may be more evident in developmental time, rather than body size alone. Third, it is possible that our larvae samples included individuals that had undergone most of their growth and as a consequence were not affected by our treatments. Finally, fat reserves may be unrelated to the diet regimes since it is likely that they are depleted during metamorphosis. Taken together our results suggest that the development of SSD in this species may be the outcome of more than one environmental variable and to some extent, although not measured by us, genetic determinants.

1. INTRODUCTION

During development, males and females are generally similar in size (i.e. vertebrates; Badyaev 2002); yet adult males and females are rarely the same size (Andersson 1994; Blanckenhorn 2005). This difference in body size is termed sexual size dimorphism (SSD).

In animals, adult body size is usually achieved *via* (i) long growth periods (Roff 1980; Stearns 1992) or (ii) fast growth rates (Badyaev 2002). If differences in these growth patterns arise between the sexes, then differences in adult body size between the sexes may follow (Badyaev 2002). For example, in species where males are larger than females, males may attain large size by growing faster or by growing for longer than females (i.e. Leigh 1992). How do sex-specific growth patterns arise? Although males and females share the same gene pool, which constrains the evolution of differential growth patterns in the sexes to some extent (see Badyaev 2002), sex-specific growth patterns are likely to be influenced by a number of mechanisms, such as sensitivity to condition during growth (Potti 1999), maternal effects (Ricklefs & Peters 1981; Kojola 1993; Cordero et al. 2001), environmental factors (Larsson & Forslund 1991; Sedinger & Flint 1991; Rhymer 1992; Saether & Heim 1993; but see Roff 1997) and time constraints imposed by seasonality (Roff 1980; Rowe & Ludwig 1991). These sex-specific differences in growth patterns are likely to evolve between populations and closely related species, resulting in the observed patterns of adult SSD (Badyaev et al. 2001; Badyaev 2002).

In insects, for example, body size at maturity will depend, to some extent, on the amount of available food during development (Blanckenhorn 1988; Emlen & Nijhout 2001) and the time constraint imposed by winter diapause (Roff 1980, 1983; Taylor 1980). Therefore, in order to achieve large body size, the development of these animals requires acceleration, in order to meet the time constraint (Blanckenhorn 1998). Achieving large size is important for both males and females since it is often linked to reproductive success at maturity (Shine 1988; Andersson 1994). Therefore, in such species, attaining

food resources during the early stages of development (i.e. the larval stage in insects) is critical (Blanckenhorn 1998).

Odonates (damselflies and dragonflies) for instance, should maximise food intake during the larval stage in order to maximise adult fitness, since growth is restricted to the larval stage (Corbet 1999). If maximising food intake during the larval stage results in large adult body size and overall good body condition (i.e. large fat reserves), males of territorial species may benefit from rich diets (i.e. Plaistow & Siva-Jothy 1999). This is because large body size is particularly advantageous for males of many territorial damselfly species (Fincke 1984; Tsubaki and Ono 1987; Serrano-Meneses et al. in press) because it often results in better territorial defense and higher mating success (compared to smaller or non-territorial males; Waage 1987; Grether 1996; Plaistow & Siva-Jothy 1999; Plaistow & Tsubaki 2000; Córdoba-Aguilar 2002; Serrano-Meneses et al. in press). The advantages of female large size, however, may be in terms of enhanced survival (Braune & Rolff 2001) or higher fecundity (Honěk 1993; Nylin & Gotthard 1998). In dragonflies, although there is ample evidence for the male benefits, the fecundity benefit for females has rarely been documented (i.e. Cordero-Rivera 1991).

As occurs with many taxa (i.e. Garel et al. 2006), research on the effect of food on larval development in odonates has been carried out in species with marked sexual seasonality. This seasonality can take up to three or four months of reproductive activity (Corbet 1999). In this situation, there is conflicting selection to attain a larger body size over a short period, which maximises development rate. This is important, since any delay will have strong effects on losing reproductive opportunities (Johansson et al. 2001; for other species of temperate invertebrates see Atkinson 1994). Animals are therefore expected to use environmental cues such as photoperiod and temperature to synchronize emergence time with the largest possible size (i.e. Frisch & Santer 2004). Indeed, with shorter periods to complete development, animals emerge with reduced size (Plaistow & Siva-Jothy 1999), which can have detrimental effects on fecundity (Johansson & Rowe 1999). Alternatively, animals may accelerate development in order to maximise body size at emergence (Strobbe & Stoks 2004). Smaller individuals are indeed observed as

the season progresses (Corbet 1999; for an exception see Rantala et al. 2001). This situation, however, has no parallel in tropical environments, since animals in these environments are usually less restricted by seasonality (Corbet 1999). This allows more relaxed pressures to exist at the time of emergence (Corbet 1999). Although animals should also compete for food, the pressure to finish larval development is less strong.

We have investigated the effect of food on the larval development of a territorial, tropical damselfly, *Hetaerina americana*. Similar to other territorial damselflies, males are larger than females in this species (Serrano-Meneses et al. in press). This species is not constrained by winter diapause, since both males and females can be found throughout the year (Peralta-Vázquez unpublished results). Animals go through a larval stage of up to several months (Corbet 1999) and a few days as tenerals, which is when they build up the necessary muscle mass and energetic reserves for territory competition. Large male body size is positively selected during territorial competition (Serrano-Meneses et al. in press) possibly because they have more fat reserves (Contreras-Garduño et al. 2006). Larger males also bear larger wing pigmented areas which are also favoured via male-male competition (Grether 1996; Serrano-Meneses et al. in press). Females visit the places males defend and soon after a female is grabbed by a male, the couple flies to other riverine places to mate and oviposit (Córdoba-Aguilar & Cordero-Rivera 2005). It is not known whether large females have an advantage for which the most obvious would be fecundity (i.e. Cordero-Rivera 1991). Paradoxically, the size of a female in this species does not correlate with the number of eggs she carries in the abdomen (Serrano-Meneses et al. in press).

Here we address two questions: (i) does diet during the larval stage have an effect on the adult size and fat reserves in both sexes? (ii) does diet regime influence larval development time as occurs in temperate dragonfly species? We have used *H. americana* larvae to answer these questions in a site where tropical conditions are prevalent.

2. MATERIALS AND METHODS

(a) Capture of larvae

242 larvae were collected at three locations (A, B, C) of the Amacuzac River in Tehuixtla, Morelos, Mexico (18° 32' 56" N, 99° 16' 23" W) between February the 9th to the 11th, 2004. Since the age of the larvae is difficult to determine in the field, we only collected larvae from intermediate to large size (from approximately 0.5 cm to 1 cm). All larvae were stored in one container with water and vegetation from the river (the vegetation was used by the larvae as substrate). After collection, the larvae were transported to the laboratory where the experiments took place. The travelling time was approximately 1 hour. In the laboratory and prior to assigning the larvae to a diet regime, the body length of every larva was measured from the tip of the head to the end of the abdomen (excluding the gills) using a digital calliper (LCD electronic digital Vernier calliper). Measurements were made to the nearest 0.001 mm.

(b) Diet regimes and change in body size

Following collection, all larvae were placed in individually labelled plastic containers with a level of water of approximately 2 cm and they were provided with a wooden stick to allow perching. To prevent emerging individuals from escaping, every container was covered with a plastic net. Water from every container was replaced with clean water every other day.

We randomly assigned each larva to one of three diet regimes. In every feeding regime the larvae were provided with three chironimid larvae at different time intervals: i) rich: fed every other day, ii) intermediate: fed every fourth day, and iii) poor: fed every sixth day. Group (i) was fed for 22±8 days; group (ii) was fed for 17±4 days and group (iii) was fed for 17±4 days. These treatments ended when the last individual in a given treatment died.

Since larval stages were unknown at the start of experiment, body size was log_{10} transformed and thus change in size from one larval stage to another was calculated as

log_{10}(size after moulting) – log_{10}(size before moulting). 95 individuals went through a single moult, whereas only two larvae moulted twice. The latter individuals were excluded from statistical analyses. Dead larvae were preserved individually in plastic tubes containing 70% ethanol.

Sexes were unknown at collection; however, post-mortem sexing was possible by noting the presence of the ovipositor in female larvae in the last stages of development. Therefore we were able to determine the sex of 102 larvae. These sexed individuals were considered in the analyses of treatments on body length, since we were interested in the effect of the diet regimes on both sexes. Sexing of emerged individuals was possible since tenerals of American Rubyspots are sexually dimorphic.

(c) Emerging individuals

After emerging, we allowed the exoskeleton of individuals to harden for approximately 2 hours. It was not possible for us to determine the exact time of emergence when individuals emerged at night; therefore we measured them approximately at 8 am (Central Standard Time) the following day. Note that this species is not active during the night (Corbet 1999; Switzer & Grether 2000); therefore it is unlikely that amount of energy reserves was substantially affected in the animals that emerged during the night. Once the individuals' exoskeletons had hardened we measured their body length using a digital calliper (to the nearest 0.01 mm). We use body length as a *proxy* for body size because other measures such as wing length and head width are highly correlated with body length (Serrano-Meneses et al. in press). These individuals were killed by twisting their heads. The fresh dead bodies were stored in silica gel to minimise humidity intake. This allowed us to accurately estimate the fat load of every individual (see below).

Fat load was measured separately for abdomen and thorax as the difference between the trait's dry weight and the weight of the trait after being treated with petroleum ether (see similar approach by Marden 1989; Serrano-Meneses et al. in press). The fat load of the abdomen plus the fat load of the thorax is referred as total fat load. This procedure was performed separately for each individual. Fat load is expressed in grams.

(d) Statistical analyses

Larvae body length between different parts of the study site was analysed using one-way ANOVA using study site as a 3-level factor. Body length during the days when larvae were collected was analysed using one-way ANOVA using date of collection as a 3-levels factor. Tukey test was used as a post-hoc test in both ANOVA's. We then tested for differences in body length between diet regimes and sex using two-way ANOVA. Body length was the dependent variable and diet regime and sex were factors. The interaction term diet regime * sex was not significant (p = 0.380), therefore it was eliminated from the model.

We tested the effect of diet regime on change in body length using two-way ANOVA with both diet regime and sex as factors. The interaction term diet regime * sex was not significant (p = 0.348), thus it was eliminated from the model. The time that the larvae spent in a given diet regime (days) was log_{10} transformed prior to analyses since it was not normally distributed (Kolmogorov-Smirnov test, p = 0.018). The time variation was then analysed using two-way ANOVA. The log_{10}(days) data was the dependent variable and diet regimes and sex were factors. The interaction term diet regime * sex was removed from the model since it was not significant (p = 0.867). The number of males and females that died or emerged during the experiment was compared using chi-squared test.

The change in size between the last larval stage and the teneral stage per sex was analysed using repeated-measures ANOVA. In this analysis body size was the dependent variable, stage (larva, teneral) was the within-subject factor and sex was the between-subject factor. Since the interaction term stage * sex was significant (see Results) we tested the differences between male and female body size at both the last larval stage and the teneral stage using independent samples t-tests.

The effect of diet regime on total fat load was analysed using Generalised Linear Model (GLM) with fat load as the dependent variable, diet regime and sex as factors and body length as a covariate. Interaction terms were not significant (p > 0.342 in all cases) so

they were removed from the analysis. To test whether males and females allocated fat differently (more fat to the abdomen or more fat to the thorax) we calculated the proportion of fat that each individual allocated to the abdomen and thorax (based on total fat load). The differential allocation per sex was analysed with paired samples *t*-tests.

The data analysed were normally distributed (Kolmogorov-Smirnov test, $p > 0.189$ in all cases) except where stated. Analyses were made using SPSS version 14.

3. RESULTS

(a) Body length at capture

Larvae body size varied across the areas where individuals were collected (one-way ANOVA: $F_{2,239} = 9.02$, $p = 0.001$; individuals collected in area A were larger than individuals collected in area B, Tukey test = 0.001). There was also variation in the size of the larvae during the different days of collection (one-way ANOVA: $F_{2,239} = 18.662$, $p = 0.001$; all groups different, Tukey test < 0.003 in all cases). Nevertheless, the size of 102 sexed larvae was not different between subsequent treatments ($F_{2,98} = 0.483$, $p = 0.618$) and sex ($F_{1,98} = 0.341$, $p = 0.561$).

(b) Changes in body size and diet regimes

Body size change was not influenced by diet regime ($F_{2,31} = 2.366$, $p = 0.111$) and was not different between the sexes ($F_{1,31} = 0.013$, $p = 0.910$). The number of days that the emerged individuals spent in a given diet regime was not different between males and females ($F_{1,37} = 0.153$, $p = 0.698$). Furthermore, although there is a trend that suggests that individuals in richer diets remained for longer in the diet regime, it is not statistically significant ($F_{2,37} = 2.730$, $p = 0.078$; Figure 1).

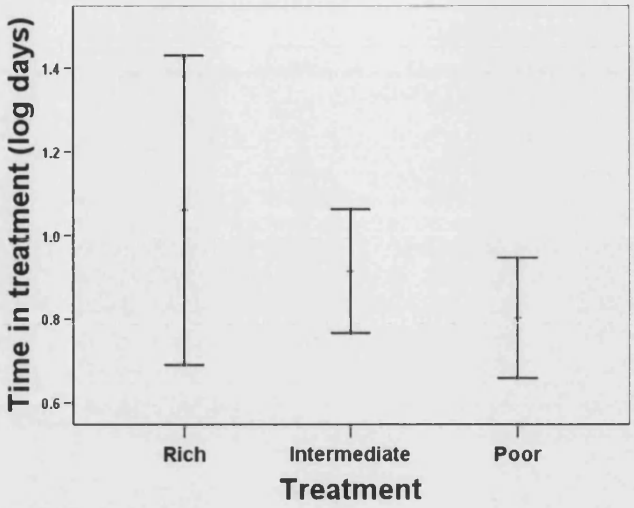

Figure 1. Time the individuals remained in each diet regime. Bars represent means ± STD. Figure is for illustrative purposes.

There was no difference in the number of males and females that died during the experiment ($\chi^2 = 0.143$, $p = 0.705$, $n = 66$). Also, the ratio of emerged males and females was not different ($\chi^2 = 0.111$, $p = 0.739$, $n = 36$).

Because of the significant interaction term, body size changes differently between the sexes from the larval to the teneral stage (stage: $F_{1,34} = 10971.808$, $p = 0.001$; sex: $F_{1,34} = 22.126$, $p = 0.001$; interaction: $p = 0.001$; Figure 2).

(c) Fat load

Diet regimes did not influence fat load ($F_{2,31} = 3.070$, $p = 0.061$). This trend was not different between sexes ($F_{1,31} = 0.025$, $p = 0.875$; Figure 3). Males allocated more fat to the abdominal cavity than to the thorax ($t_{18} = 2.956$, $p = 0.008$). Females show a similar trend to males although it was not statistically significant ($t_{16} = 1.769$, $p = 0.096$).

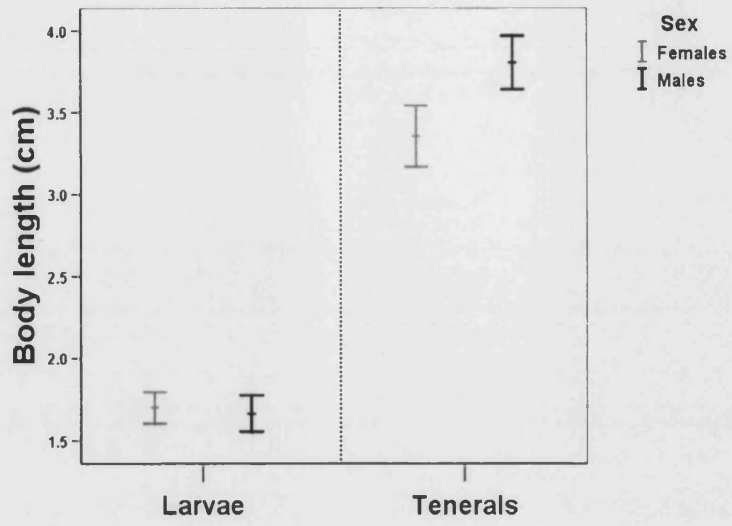

Figure 2. Size of the individuals measured as both larvae and as tenerals. Bars represent means ± STD. Males and females do not differ in size at the last larval stage ($t_{34} = 0.994$, $p = 0.327$), but they have different sizes at the teneral stage ($t_{34} = 7.707$, $p < 0.001$) as measured approximately 2 hours after emergence.

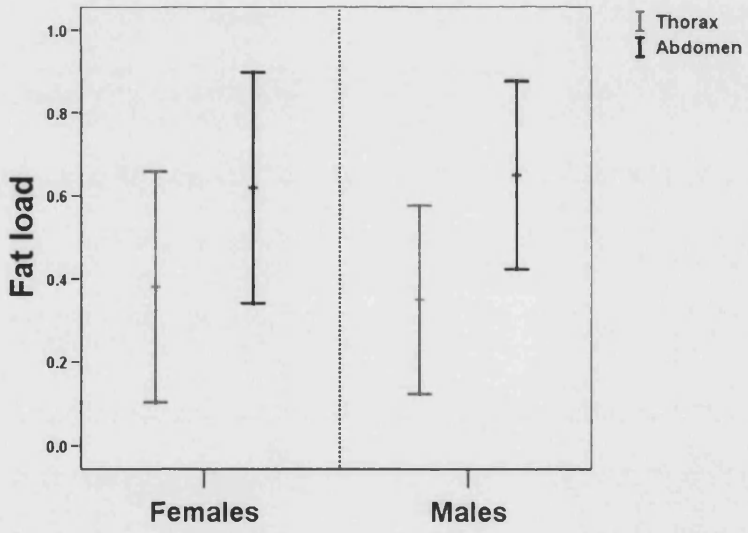

Figure 3. Allocation of body fat in emerging males and females. Units are proportions. Bars represent means ± STD.

4. DISCUSSION

Variation in the reproductive success of male (Fincke 1982; Banks & Thompson 1985; Koenig & Albano 1987; McVey 1988; Moore 1989; Córdoba-Aguilar 2002; Serrano-Meneses et al. in press) and female odonates (Banks & Thompson 1987) has been documented; nevertheless, these studies have been limited to sexually mature individuals. Little is known about the survival of individuals from emergence to sexual maturity and whether it is correlated to condition at emergence (Corbet 1999). In one experiment, Anholt (1991) manipulated both the density of individuals and food availability in an experimental population of larvae of the non-territorial damselfly *Enallagma boreale*. Note that at sexual maturity, females are considerably heavier than males (Anholt 1991), similar to most non-territorial damselflies (Anholt et al. 1991). By inducing competition between the larvae over scarce resources, Anholt (1991) obtained small body size in the emerging individuals, whereas body size was generally larger in those individuals emerging from treatments with low densities and high resources. Large body size was related to increased survival from emergence to sexual maturity in both males and females during years of favourable environmental conditions (see Anholt 1991). Large body size at sexual maturity, on the one hand, was generally disadvantageous for males. Males of intermediate and small size, therefore, were observed to obtain most copulations. This has also been observed in males of the closely related, non-territorial *Enallagma hageni* (Fincke 1988). On the other hand, large female size was not associated with higher reproductive success (Anholt 1991): female body size was found to be under stabilising selection.

The American Rubyspot exhibits male-biased SSD at sexual maturity (Serrano-Meneses et al. in press). Large male size has been shown to be advantageous for males because it is related to territorial defence and mating success (Serrano-Meneses et al. in press). However, to our knowledge, nothing is known about the advantages of a given female body size. As a general rule, male size should be advantageous for males of territorial species in damselflies (Anholt et al. 1991; Serrano-Meneses et al. unpublished results). In this work we asked whether, similar to other odonates (Fincke 1988; Anholt 1991;

Plaistow & Siva-Jothy 1999), larval diet was likely to influence the developmental onset of adult body size and hence SSD. Our treatments did not influence size change in either larval stadia or emerged individuals. This result is not the product of differences in the initial body size across the treatments, since at the beginning of the experiments body size was distributed similarly across treatments. This result, therefore, may have three possible explanations. First, it is possible that food availability alone does not influence body size in this species. Instead, the determination of adult body size may be the outcome of interplay between genetic and other environmental variables (see Badyaev 2002), such as temperature or larval competition over resources (Anholt 1991; Corbet 1999). Second, the effect of larval nutrition may be more pronounced on developmental time (Corbet 1999). This could be the case in species in which tropical conditions are prevalent throughout the year, such as our study site. Note that this species is not affected by winter diapause and that sexually mature individuals can be found throughout the year. Therefore an effect of larval nutrition on developmental time can be expected since favouring environmental conditions are prevalent in these areas, causing the individuals to experience less pressure to emerge before the end of the reproductive season (Blanckenhorn 1998; Crowley & Johansson 2002). Without high pressure to emerge early, larvae could undergo long developmental times and feed for longer in order to achieve large size (i.e. Crowley & Johansson 2002). This is possible since most odonates do not have a fixed number of stadia (Corbet 1999). On the one hand, larvae tend to undergo more stadia when favouring conditions (such as high food availability) are present (Corbet 1999). On the other hand, larvae may emerge earlier when conditions are not favourable (Corbet 1999), even if large body size at emergence will not be achieved. This was not supported by our data; however, this could be caused by the low sample sizes that resulted from high mortality rates across the diet regimes. Finally, since we could not determine the larval stage of individuals, it is possible that our sample consisted mainly of larvae that were already at an advanced stage of development (near emergence). Therefore the body length criteria (see Materials and Methods) we used when selecting larvae may have been too large and as a consequence had already undergone most growth.

Our results on body fat allocation suggest that fat load in the emerged individuals was not influenced by a particular diet regime. This may suggest that body fat is unrelated to diet regime. However, it is also possible that the accumulation of fat reserves prior to emergence is depleted during metamorphosis (Corbet 1999). In this scenario, the emerged tenerals would have to feed for a number of days in order to accumulate fat resources that will later be used during territorial contests (i.e. Anholt 1991). Indeed recently emerged individuals have been shown to double in mass after a foraging period of one to three weeks (Anholt et al. 1991), suggesting that it is during the teneral, pre-reproductive period that animals accumulate the fat reserves that will later be used during territorial defence by males and destined for egg production by females (Corbet 1999; Plaistow & Tsubaki 2000). We suggest that this scenario is likely to occur in American Rubyspots, providing an explanation for the lack of a relationship between larval diet regimes and teneral fat content.

Finally, larvae did not exhibit SSD whereas adult males were significantly larger than females. We explored the pattern of size change (from larval to teneral size) between the sexes. Our results show that SSD arises only after metamorphosis. The absence of SSD during development had not been documented previously in this species, whereas the extent and direction of adult SSD is consistent with field observations in this species (Serrano-Meneses et al. in press) and with the prediction of the direction of SSD in territorial damselflies (Serrano-Meneses et al. unpublished results). Why is there a lack of SSD in larvae? One explanation is that males gain survival benefits from being size-monomorphic, since male-biased SSD during the larval stage is associated high mortality rates that result from high foraging effort (Stoks & Johansson 2000). Therefore it is possible that males are prevented from being large during the larval stage because of its mortality costs. Large male size, therefore, may only be advantageous for adults, since it is associated to mating success (i.e. Moore 1990; Serrano-Meneses et al. in press).

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Chapter 4. *The development of sexual differences in body size in Odonata*

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Abstract

Adult body size is the result of important environmental, maternal and/or genetic effects acting on animals during development. Here we investigated how sexual size dimorphism (SSD) develops in seven species of Odonata: *Anax imperator, Cordulegaster boltonii, Onychogomphus uncatus, Oxygastra curtisii* (Anisoptera), and *Cercion lindeni, Ischnura graellsii and Platycnemis acutipennis* (Zygoptera). We measured SSD both in the last larval and adult stage in the same individuals, which were reared in laboratory conditions. Our aims was to see whether SSD in adults was already present and in the same direction in the larval stage and if it correlated with mating system (e.g. males of territorial species being larger than females; with no clear pattern for nonterritorial species). We found that although larval differences in size may be present, these are not necessarily shown in the adult stage (they may change, disappear or even get reversed). Also, mating system was not related to patterns of adult SSD. Differences in SSD in larvae may be caused by differential use of resources via niche differentiation. We highlight the fact that fecundity selection that selects for large females may be acting behind the observed patterns in SSD in adults.

1. INTRODUCTION

Differences in body size between males and females are common in many adult animals (Andersson 1994). For instance, in many invertebrate species, sexually mature females are several times larger than males (female-biased SSD), whereas in most mammals and birds, males are the larger sex (male-biased SSD; Abouheif & Fairbairn 1997; Blanckenhorn 2005; Székely et al. in press). The degree and direction of SSD in a given species or taxa are usually explained by i) fecundity selection acting on female body size that usually produces female-biased SSD through increasing fecundity with female size (Honěk 1993; Head 1995; Prenter et al. 1999), ii) differential niche utilisation between males and females reducing the competition for resources between the sexes and promoting morphological divergence (Selander 1966; Shine 1989; Thom et al. 2004), and iii) sexual selection favouring male-biased SSD in species in which males compete over females on the ground (Anderson & Fedak 1985; Lindenfors & Tullberg 1998; Serrano-Meneses & Székely 2006), or female-biased SSD in species in which males compete in the air (Andersson & Norberg 1981; Raihani et al. 2006; Serrano-Meneses & Székely 2006).

Studies often approach SSD from a functional perspective to investigate the ecological and/or behavioural causes and consequences of SSD in adults (see Blanckenhorn 2005). However, the observed patterns of SSD in the adult stage are the result of important differences in physiology, behaviour, and ecological niches between the sexes during growth and development (Le Gaillard et al. 2006). These differential factors may produce divergent selective pressures on the ontogeny of males and females (i.e. developmental time, size at hatching or growth rate) leading to SSD in adults (Badyaev, 2002). Thus, adult body size may be genetically fixed (i.e. queen size in the ant *Leptothorax rugatulus*; Rüppell et al., 2001), or influenced by environmental factors, such as the amount of available food during the early stages of development (i.e. male body size in the horned beetle *Onthophagus taurus*; Emlen & Nijhout 2001). Note, that although the long-term evolution of SSD is to an extent constrained by genetic factors

(Reeve & Fairbairn 1996), ecological or behavioural pressures can drive the evolution of SSD even in presence of strong genetic correlation between the sexes (Badyaev 2002).

The order Odonata is an example of taxa with marked differences in SSD. Several species have been intensively studied in terms of sexual behaviour and, broadly speaking, two mating systems have been clearly outlined: territorial and nonterritorial species (Corbet 1999). In the former, males usually gather at aquatic sites defending a place against male conspecifics, where females arrive to mate and lay eggs (i.e. Waage 1973). In the latter, males do not defend territories but look for females in a scramble competition manner (i.e. Fincke 1985). These two mating system types should be correlated with different ranges of SSD (for a study suggestive of this claim see Johansson et al. 2005): in territorial species, males should be the larger sex, while in nonterritorial species, this prediction would not hold as fecundity selection pressure may have as a consequence similar sizes and/or female biased SSD. How these differences develop in the ontogeny, nevertheless and to our knowledge, have been scarcely studied (i.e. Mikolajewski et al. 2005).

Adult body size usually develops in one of two ways: organisms may grow at a faster rate, or gradually over a longer period (Blanckenhorn 2005). Therefore, differences in adult body size between the sexes are likely to arise if the sexes differ in developmental time and/or growth rate. In many insects, such as odonates, these ontogenic factors are not entirely determined genetically, but they are likely to fluctuate with changes in environmental conditions such as temperature, time in the reproductive season and available food (Corbet 1999). Food, in particular, is a limiting resource to accrue maximum fitness benefits in both sexes. On the one hand and for example, in territorial dragonflies males whose larval development has been provided with rich diets gain a large size, more muscle mass and fat reserves at emergence (Plaistow & Siva-Jothy 1999). These traits are related to male-male competition for mating territories since large individuals with large fat reserves are more successful during aggressive territorial contests (Marden & Waage 1990; Plaistow & Tsubaki 2000; Serrano-Meneses et al. in press). Since territorial males often gain more matings than non-territorial individuals

(i.e. Waage 1973; Córdoba-Aguilar 2000; Grether 1996), the former obtain higher paternity benefits compared to the latter. Females, on the other hand, gain fitness benefits via an enhanced survival at emergence (Braune & Rolff 2001) and expected higher fecundity via a large size (Cordero-Rivera 1991).

Here we investigate the development of SSD in five territorial and two non-territorial odonate species (Anisoptera and Zygoptera). We have looked at both the last larval and the adult stage measured in the same individual and reared in the laboratory. On the one hand, we investigated whether SSD in adults is correlated with SSD of last larval stage prior to adult emergence. For instance, if in species with male-biased adult SSD, male larvae may be already larger than the female larvae. On the other hand, we expect that this dimorphism will be related to the mating system: in territorial animals, males should exhibit larger size than females while this pattern should not hold necessarily for nonterritorial species. Finally, we investigated whether patterns of SSD in the larval stage predict future patterns in the adult stage to see whether a trend is held during ontogeny.

2. MATERIALS AND METHODS

(a) Larvae

The larvae of seven species (Anisoptera: *Anax imperator*, *Cordulegaster boltonii*, *Onychogomphus uncatus*, *Oxygastra curtisii*; Zygoptera: *Cercion lindeni*, *Ischnura graellsii*, *Platycnemis acutipennis*) were collected in 4 sites in the province of Pontevedra, North-West Spain (see Table 1 for mating systems, dates and collection sites). We chose these species since their biology is well known (Corbet 1999), and they are commonly found throughout North-West Spain. Since we were interested in the extent and direction of SSD at the last larval stage (F0 henceforward) and at adulthood, we only collected those larvae that were presumed to be near emergence. At collection, the sex of the larvae was noted using the presence or absence of the ovipositor. All larvae collected in a given day were stored in one container with water and vegetation

from the river (the vegetation was used by the larvae as substrate). The larvae were determined in the field using the taxonomic key of Gerken & Sternberg (1999). The larvae were then transported to the laboratory at University of Vigo within one hour of collection where the rearing of the larvae took place. Before assigning the larvae to individual rearing containers, we measured the body length of every larva (from the tip of the head to the end of the abdomen, excluding the gills in the case of zygopterans) using a digital calliper (LCD electronic digital Vernier calliper; measurements made to the nearest 0.001 mm). Larvae in F0 stadium were identified from the advanced development of the wing sheaths and eye index (Corbet 1999), whereas larvae in other stadia were returned to their natural habitat (see Table 1).

Table 1. Locations and dates of collection. Mating system denotes territorial (T) or non-territorial (NT) species. Initial *n* is the number of larvae collected for a given species (the number of emerged adults differs to these due to mortality). Rearing duration refers to a period in days which covers the day when the larvae were captures until the tenerals emerged.

Anisoptera						
Species	Mating system	Site	Coordinate	Date of collection	Rearing duration in days	Initial *n*
Anax imperator	T	Marcosende	42° 4' 4" N, 8° 40' 4" W	12-May-06	48	30 ♂ 30 ♀
Cordulegaster boltonii	T	Covelo	42° 23' 10" N, 8° 31' 20" W	29-Apr-06	50	30 ♂ 30 ♀
Onychogomphus uncatus	T	Covelo	42° 23' 10" N, 8° 31' 20" W	29-Apr-06	18	30 ♂ 30 ♀
Oxygastra curtisii	T	Covelo	42° 23' 10" N, 8° 31' 20" W	29-Apr-06	31	30 ♂ 30 ♀

Table 1 continued.

Zygoptera						
Species	Mating system	Site	Coordinate	Date of collection	Rearing duration in days	Initial *n*
Cercion lindeni	T	Marcosende	42° 4' 4" N, 8° 40' 4" W	12-May-06	18	20 ♂ 20 ♀
Ischnura graellsii	NT	Lourizán	42° 25' 24" N, 8° 40' 40 " W	25-May-06	50	35 ♂ 35 ♀
Platycnemis acutipennis	NT	Tenorio	42° 28' 50" N, 8° 37' 40" W	05-Nov-05	67	20 ♂ 20 ♀

Following measurements all larvae were placed in individually labelled plastic containers with a wooden stick to allow perching, and appropriate water volume that filled approximately 2.5-3.5 cm of container for zygopterans and anisopterans, respectively. Each container was then covered with a plastic net to prevent emerging individuals from flying. Water was replaced by fresh water every day. Zygopterans were fed daily with 1 worm (either *Tubifex tubifex* or *Lumbriculus variegatus*), while anisopterans were fed with 2 worms (Van Gossum et al. 2003). The diet for each species and sex was such that no individual was fed on a single species of worm: zygopterans were fed every day with a different worm and anisopterans were fed daily with one worm of each species.

Emerging tenerals were placed in individual insectaries of approximately 60 cm x 50 cm x 50 cm. Inside each insectary eight wooden sticks were provided as perching substrate, and a water container covered with a plastic net was provided to maintain humidity. The natural photoperiod in the appropriate season was maintained (approximately 12 hours) by illuminating the insectary with a commercially available light bulb. We allowed each individual approximately 24 hours in an insectary until his/her exoskeleton was fully hardened, and then measured their body length using a digital calliper. After this, we either released the adults in the location of capture, or those species that emerged during winter (*P. acutipennis*) and were not likely to survive in nature were stored in absolute ethanol.

(b) Statistical analyses

We only included those individuals in the analyses that survived until emergence. We used mixed-model ANOVA in which body size was the dependent variable, developmental stage (F0, adult) was the within-subject factor and sex (male, female) was the within-subject factor. We also included the interaction term stage * sex in the models.

To compare the changes in SSD in the two stages and between sub-orders, first we calculated SSD for larvae (SSD_{F0}) and adults (SSD_{adult}) separately as Log_{10} (male size) – Log_{10} (female size) (Smith 1999), and then calculated SSD_{delta} = SSD_{adult} — SSD_{F0}. Thus a positive SSD_{delta} indicates shift toward male-biased SSD, whereas a negative SSD_{delta} indicates shift toward female-biased SSD.

Statistical analyses were carried out using SPSS Ver. 14. Results are provided as mean ± STD.

3. RESULTS

(a) Anisoptera

In three out of the four territorial species SSD was statistically significant during F0, whereas in one species SSD developed at the adult stage (Table 2; Figure 1a). Two out of four species exhibited female-biased SSD at F0 (*A. imperator*, *C. boltonii*,) whereas *O. curtisii* was monomorphic. SSD only appeared in the adult stage in *O. uncatus*, in which females were larger than males. A statistically significant interaction between stage and sex indicated that the magnitude of SSD changed from F0 to adults in *C. boltonii* and *O. uncatus* (Tables 2 & 3).

(b) Zygoptera

In the territorial species *C. lindeni* adult male-biased SSD was apparent in the larval stage. In the other two non-territorial species adult SSD was monomorphic in the adult

stage, whereas in the larval stage one showed female-biased SSD (*P. acutipennis*) and *I. graellsii* exhibited no SSD in either adult and F0 stages (Table 2; Figure 1b). A statistically significant interaction between stage and sex indicated that the magnitude of SSD changed from F0 to adults in *C. lindeni* and *P. acutipennis* (Tables 2 & 3).

Table 2. Mixed model ANOVAs of body length (response variable) in relation to stage (within-subjects factor; F0, adult) and sex (between-subjects factor; male, female). A different model was built for each species.

Anisoptera

Species	Error	Stage		Sex		Stage * sex	
	d.f.	*F*	*p*	*F*	*p*	*F*	*p*
Anax imperator	17	872.893	0.001	19.509	0.001	4.103	0.059
Cordulegaster boltonii	18	13745.35	0.001	557.615	0.001	435.321	0.001
Onychogomphus uncatus	18	5758.845	0.001	8.054	0.011	26.123	0.001
Oxygastra curtisii	19	4029.65	0.001	0.203	0.657	0.056	0.816

Zygoptera

Species	Error	Stage		Sex		Stage * sex	
	d.f.	*F*	*p*	*F*	*p*	*F*	*p*
Cercion lindeni	22	7215.418	0.001	10.381	0.004	5.657	0.026
Ischnura graellsii	24	1418.217	0.001	0.066	0.799	1.340	0.259
Platycnemis acutipennis	22	9357.662	0.001	0.012	0.913	12.529	0.002

Table 3. SSD in odonate larvae (F0 stage) and adults. > and < indicate statistically significant differences in size between the sexes, whereas = indicates non-significant difference. In brackets we provide SSD_{delta} (see Methods). A positive SSD_{delta} indicates shift toward male-biased SSD, whereas a negative SSD_{delta} indicates shift toward female-biased SSD. T and NT indicate whether the species is territorial or nonterritorial respectively.

		SSD in adults		
		Males > Females	Males = Females	Males < Females
SSD in F0	Males > Females	*Cercion lindeni* (-0.001) (T)		
	Males = Females		*Ischnura graellsii* (0.046) (NT) *Oxygastra curtisii* (0.006) (T)	*Onychogomphus uncatus* (-0.036) (T)
	Males < Females		*Platycnemis acutipennis* (-0.056) (NT)	*Cordulegaster boltonii* (-0.056) (T) *Anax imperator* (0.046) (T)

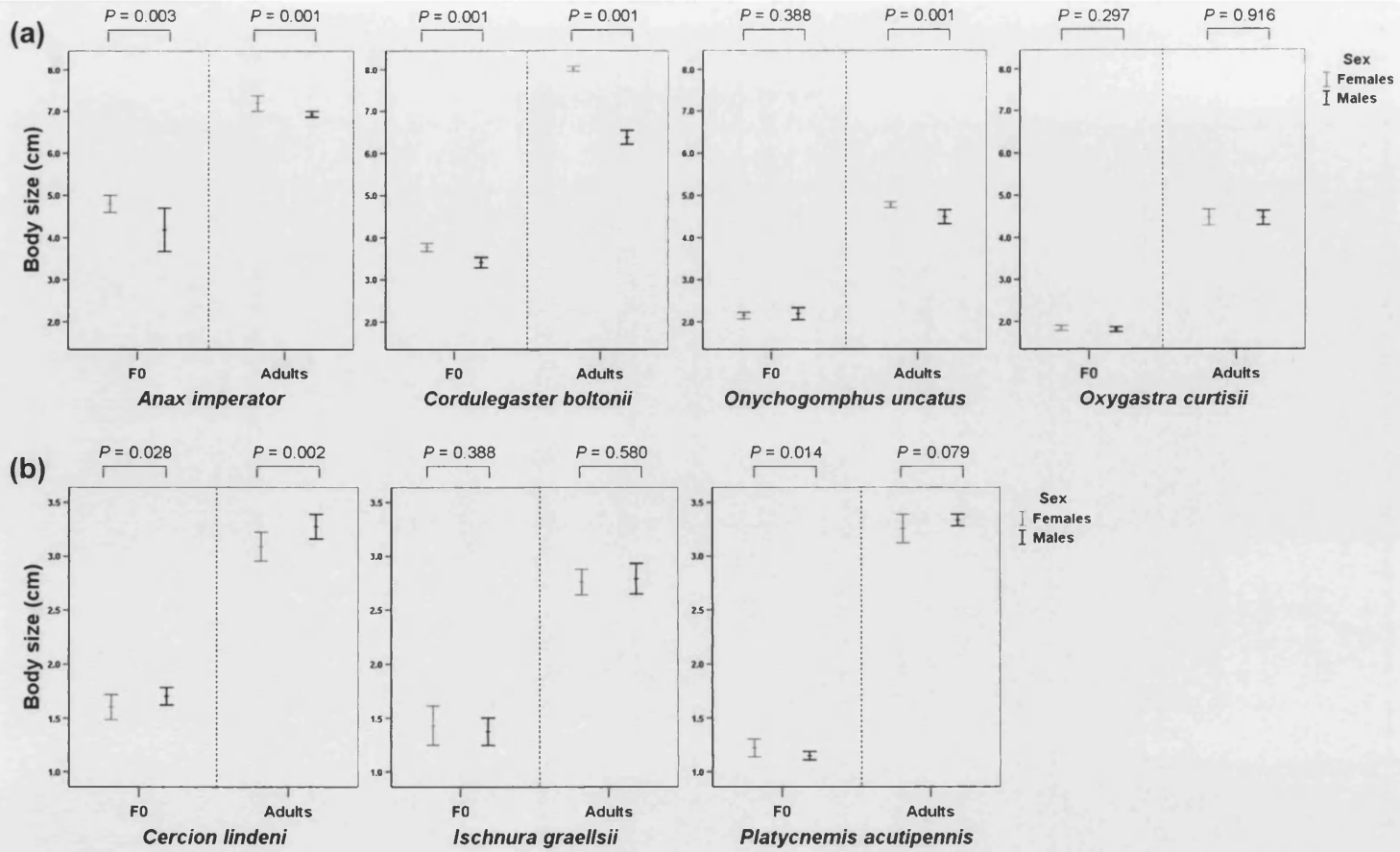

Figure 1. Body length of F0 larvae and adults in (a) Anisoptera and (b) Zygoptera (means ± STD). *p* indicates the probability value of independent *t*-tests.

4. DISCUSSION

We observed SSD in most species examined. In three cases, the dimorphism shown by adults was already present in the larval stage, in two cases both larvae and adults did not differ with two cases in which the pattern observed in the larval stage changed when adults. With this, it is difficult to advance the idea that the size differences observed in the adult stage are similar to prior stages. This is corroborated by the lack of clear predictable SSD patterns in the larval that can be translated to the adult stages.

We predicted that in territorial species, there should be a biased SSD with strong sexual selection acting on males as body size is favoured via male-male competition. In this type of competition, elements such as muscle mass and fat reserves are positively selected (i.e. Marden & Waage 1990; Plaistow & Siva-Jothy 1999; Plaistow & Tsubaki 2000) and the larger the animal, the greater these elements (Serrano-Meneses et al. in press). On the other hand, in the absence of male-male competition mediated by physical encounters, fecundity selection may act extensively resulting in no SSD at all or female-biased SSD. Except for one species (*C. lindeni*), in all territorial species examined in this paper, we did not find that males were larger than females in the adult stage. In fact, in three anisopterans, females were larger than males at this stage. In two of these species, this difference was in fact already present in the larval stage. Several explanations may be brought about. The first is that despite being territorial, males are not larger possibly for reasons related to the factors that females face that may compensate differences in SSD. One factor may be fecundity selection on which females would tend to become larger. This may be a strong force in those species in which females are larger and even in the only territorial species which did not show SSD (*O. curtisii*). The difference in size may be already present in the larval stage although some other explanations may be put forward too. One explanation is that there are ecological differences between the sexes prior to emergence. To date we know of no evidence that can suggest differences of habitat use between the sexes but partially this is because there are no published accounts or research related to this. It may be a promising avenue of research. Although some form of territoriality which includes aggressive behaviour, has been shown in the

larval stage of several odonates (i.e. Rowe 2004). Whether this behaviour is sex-specific has not been documented.

On the other hand, in the two non-territorial species, there were no differences in SSD at the adult stage and one showed female biased SSD in the larval stage (*P. acutipennis*). Again, it may be that fecundity selection may be behind this absence of SSD although it is interesting that females are larger in the larval stage. It may simply be that females have already developed larger as emergence is soon to occur although, but it cannot be that natural selection is operating in the larval stage differentially. Recent evidence in *Ischnura elegans*, for example, indicated that males developed faster and, consequently, smaller than females presumably due to selection that scramble competition may produce (Abbot & Svensson 2005; for a similar result also see Mikolajewski et al. 2005). This means that males are under strong selection to emerge to look for females. Although this mechanistic explanation may as well apply to territorial species, the fact is that indeed the sexes may show differences in the larval stage which may explain our results. Another recent set of results using nine libelullids that included territorial and nonterritorial species, did not show sexual differences in egg size (Schenk & Söndgerath 2005). This suggests that the presumable SSD in Odonata does not start in the egg stage but takes place during larval development. Mikolajewski et al (2005) for example documented that *Coenagrion puella* males showed greater activity than females in the larval stage (for an example of no differences in activity between the sexes see Stoks 1999). In this non-territorial species and in the same study, females were larger and had more mass than males at emergence. This pattern of sexual differences is similar to what we have shown in *P. acutipennis* which is also non-territorial.

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Chapter 5. *Sexual selection, sexual size dimorphism and Rensch's rule in Odonata*

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Manuscript

Details and extent of the contributions by authors

M. A. Serrano-Meneses: collected the majority of data on body size and mating systems, construction of the phylogeny, phylogenetic analyses, manuscript writing (60%)

A. Córdoba-Aguilar: general editing of the manuscript, suggestions to improve the study (10%)

T. Székely: general editing of the manuscript, suggestions to improve the study (10%)

M. Azpilicueta-Amorín: data collection (10%)

E. González-Soriano: provided data on body size for several species that can be found in his private collection (10%)

Abstract

Odonata (damselflies and dragonflies) exhibit a range of sexual size dimorphism (SSD) that includes both male-biased (males > females) and female-biased SSD (males < females). Here we use phylogenetic comparative analyses to investigate the influences of sexual selection on SSD. Firstly, we show that odonates exhibit a relationship between body size and SSD that is consistent with Rensch's rule. Interestingly however, when Anisoptera and Zygoptera are analysed separately, only Zygoptera exhibit Rensch's rule whereas the sizes of males and females are scaled isometrically in Anisoptera. Secondly, we test the influence of territoriality and agility on SSD using generalised least squares and show that male-biased SSD increases with territoriality in Zygoptera, but not in Anisoptera. A measure of wing shape thought to be correlated with male agility is not related to SSD. Taken together, our results suggest that sexual selection has to some extent shaped the evolution of SSD in Odonata, however, other evolutionary processes such as ecological and/or environmental influences cannot be excluded.

1. INTRODUCTION

Sexual size dimorphism (SSD), a difference in body size between males and females, is a prevailing characteristic of many animals (Andersson 1994). SSD can vary both in direction and degree. For example, in some mammals and birds, the male can be up to five times larger than the female, whereas in some fish, spiders and marine invertebrates, females can be hundreds of times larger than the males (Blanckenhorn 2005) or, in some extreme cases, even tenths of thousands heavier (Norman et al. 2002).

Three major selective processes may explain such differences in size. First, SSD may arise if one sex competes more intensely over mates than the other (Webster 1992; Owens & Hartley 1998). However, the direction of SSD usually depends on whether the most competitive sex (usually the males) displays or competes on the ground, or in the air (Payne 1984; Jehl & Murray 1986; Figuerola 1999; Székely et al. 2004). On the one hand, sexual selection usually favours large male size relative to female size, *via* male-male competition or female choice, when males compete or display on the ground (Anderson & Fedak 1985; Lindenfors & Tullberg 1998; Székely et al. 2000; Lindenfors et al. 2002). On the other hand, small male size is favoured when males compete or display in the air (Andersson & Norberg 1981; Hakkarainen et al. 1996; Blomqvist et al. 1997; Székely et al. 2000; Raihani et al. 2006; Serrano-Meneses & Székely 2006), when small male size enhances male agility. Thus, selection for small, agile males is expected to produce female-biased SSD.

Second, SSD may be shaped by ecological processes. For example, if resources are scarce, the sexes may adapt to the exploitation of different resources in order to reduce or avoid competition (Selander 1966; Shine 1989; Thom et al. 2004). This differential exploitation of resources, in the long term, is expected to drive further morphological differentiation between the sexes. Alternatively, in areas of low productivity, sexual selection for increased body size may be constrained due to low availability of resources (Blomqvist et al. 1997; Colwell 2000). Note, however, that under these circumstances,

the direction of SSD is not predictable so that either males or females can be the larger sex.

Third, fecundity selection for large female size may account for female-biased SSD (Andersson 1994). This is likely to be the case if female fecundity (i.e. clutch size, egg size, egg production, offspring quality) increases with her body size (Darwin 1871; Wootton 1979; Shine 1988; Honěk 1993; Preziosi et al. 1996), and if fecundity selection on female body size is stronger than sexual selection acting on male size (i.e. spiders; Head 1995; Prenter et al. 1999).

A wide range of animal taxa including birds (Colwell 2000; Raihani et al. 2006; Serrano-Meneses & Székely 2006), lizards (Cullum 1998), turtles, primates and water striders (Abouheif & Fairbairn 1997; Fairbairn 1997), exhibit an allometric relationship between body size and SSD that is known as Rensch's rule (Rensch 1950; Abouheif & Fairbairn 1997; Fairbairn 1997). In these taxa, consistently with the notion of Bernhard Rensch (1950), SSD increases with body size in species in which males are larger than females, whereas SSD decreases with body size in species in which the females are the larger sex. There are several potential explanations for Rensch's rule (Fairbairn 1997). However, the only explanation that is currently supported by the data is sexual selection: using comparative analyses Székely et al. (2004) showed that Rensch's rule in shorebirds is driven by different aspects of sexual selection: the intensity of male-male competition, the agility of male displays and the statistical interaction between male-male competition and display agility.

Odonata (dragonflies Anisoptera, and damselflies Zygoptera) is an excellent group for testing functional hypotheses of SSD. This insect order exhibits both male-biased SSD (i.e. *Orthemis ferruginea*, *Hetaerina americana*; pers. obvs.) and female-biased SSD (i.e. *Dromogomphus spinosus*, *Enallagma antennatum*; pers. obvs.), sometimes, within a single genus (i.e. *Libellula croceipennis* and *Libellula herculea*; pers. obvs.). What may be the functional explanation for such diversity in SSD? In a comparative study, Anholt et al. (1991) showed that females are heavier than males in most non-territorial

odonates; nevertheless the difference in body mass between the sexes is less apparent or even the opposite in territorial odonates. However, Anholt et al. (1991) did not control for the phylogenetic non-independence of species (Harvey & Pagel 1991), thus their results may be biased at best, or false at worst.

Odonates (Anisoptera and Zygoptera) are excellent organisms to test functional explanations of SSD. First, they exhibit a range of SSD that spans from female-biased dimorphism to male biased dimorphism (Anholt et al. 1991; Andersson 1994). Second, their mating systems are highly variable between and within species (Corbet 1999): some species have territorial males that patrol and defend sites where the females oviposit, whereas others exhibit scramble male-male competition. Third, sexes are straightforward to identify (Corbet 1999), whereas in many beetles, butterflies and moths genitalia inspection is necessary to distinguish males from females. Fourth, the adults attain their full size at emergence and they don't grow afterwards (Anholt et al. 1991; Fincke et al. 1997; Serrano-Meneses et al. in press), so that measurements at emergence describe adequately the size of adult males and females. Finally, phylogenetic relationships between genera and family are reasonably understood (i.e. Misof et al. 2001; Carle & Kjer 2002; Rehn 2003; Dumont et al. 2005), although the current phylogenetic hypotheses, as for many organisms, may need to be revised in the light of new molecular and morphological data.

Johansson et al. (2005) recently investigated SSD and sex ratios in Odonata. Using exuviae hind tibia length, Johansson et al. (2005) showed that Odonata follows Rensch's rule. This study, however, has not tested a functional explanation of SSD and was limited to 21 species (15 Anisopterans, 6 Zygopterans)

We have four major objectives in this study. Firstly, to establish the distribution of SSD in adult Odonates using 133 species (63 anisopterans and 70 zygopterans). Secondly, to test functional explanations of SSD using a recent phylogenetic comparative method, generalised least squares. Specifically, we investigate whether territoriality and male agility predict SSD. Thirdly, we test whether Odonates exhibit allometry consistent with

Rensch's rule. Finally, we test two candidate explanations of allometric relationship in body size: territoriality and male agility. Taken together, these analyses are the most comprehensive tests of SSD in odonates to date.

2. MATERIALS AND METHODS

(a) Data and SSD

We collected data on body and wing length of males and females from the Odonata collection of the Natural History Museum, London (United Kingdom), from natural populations in Spain, Mexico and Finland and from published sources (see Appendix 1). Data on territoriality were compiled from published sources (see Appendix): non-territorial species were scored zero, and territorial species (i.e. resource or site defence) were scored one. We also photographed the extended wings of one male for each species at the Natural History Museum using a digital camera (Canon 20D with a 60mm Macro lens) from a constant distance (0.5 m). We set the following criteria to include a species in our dataset: (i) data on body length body length should be available for at least three individuals for both sexes, and (ii) phylogenetic hypothesis was available.

We use body length as a *proxy* for body size for two reasons. Firstly, body length and wing length are highly correlated both in males ($r = 0.913$, $P = 0.0001$, $n = 133$ species) and females ($r = 0.928$, $P = 0.0001$, $n = 133$ species). Secondly, body length is invariable with age whereas body mass may vary with age and condition of individuals (Anholt et al. 1991; Grabow & Rüppell).

Male and female body size were log_{10} transformed prior to analyses, and SSD was calculated as log_{10} (male body size, in cm) – log_{10} (female body size, in cm). In an overview of SSD indices, Smith (1999) concluded that this measure is one of only two preferred indices of SSD, since *log* differences tend to be symmetric around zero, and less likely to violate the assumptions of parametric tests.

(b) Male agility

Data on male agility were not available for vast majority of species, so we decided to use *a proxy* measure based upon wing shape. Wing shape often reflects adaptation to life style. For instance, migratory birds have pointier and more convex wingtips than non-migratory birds (Lockwood et al. 1998).

We estimated male agility from wing area asymmetry: slower but more manoeuvrable species have low proximal wing areas relative to distal areas, while faster and less manoeuvrable species have high proximal wing areas (Grabow & Rüppell 1995; Wakeling 1997). On the digital photographs we measured the distance in pixels from the distal tip of the wing to the insertion to the thorax, and we divided the wing into two areas (proximal and distal) using the midpoint of wing length. Using ImageJ 1.34s (National Institutes of Health, http://rsb.info.nih.gov/ij/) we measured the total area in pixels of the wing, as well as the area in both proximal (*P*) and distal (*D*) parts of the wing. We then estimated the proportion of the wing area in the proximal side of the wing as $P / (P + D)$. This process was repeated for each wing, and the mean of four *P*s were calculated for each male. Note that small proportions (< 0.5) reflect low proximal areas and therefore more manoeuvrability, whereas high proportions denote larger proximal wing area and thus adaptation for fast flight.

(c) Phylogeny

We use a composite phylogeny of 16 families and 133 species (Figure 1), since no single comprehensive phylogenetic hypothesis is yet available that would include most (or all) species. Our phylogeny was built using the morphology-based phylogram of the high-level relationships of Odonata (Rehn 2003, Figure 6) as augmented by recent molecular phylogenies of families and genus. Thus Aeshnidae, Chlorogomphidae, Cordulegastridae, Corduliidae, Gomphidae and Petaluridae were taken from Misof et al. (2001), and Libellulidae from Carle and Kjer (2002). Calopterygidae, Chlorocyphidae, Euphaeidae, Hetaerinidae, Megapodagrionidae and Platycnemididae were taken from Dumont et al. (2005); *Enallagma* (Coenagrionidae) was taken from Brown et al. (2000) and *Ischnura* (Coenagrionidae) was taken from Chippindale et al. (1999). Lestidae

(*Lestes disjunctus* and *Lestes viridis*), Pseudostigmatidae (*Megaloprepus caerulatus* and *Pseudostigma aberrans*) and *Argia* (Coenagrionidae, *Argia plana* and *Argia sedula*) were augumented to the phylogeny according the position of the corresponding family or genus in the phylogram of Rehn (2003).

(d) Phylogenetic analyses

For testing the Rensch's rule, we used the phylogenetic independent contrasts method of Felsenstein (1985) as implemented by CAIC (Purvis & Rambaut 1995) to control for the phylogenetic non-independence of species (Harvey & Pagel 1991). An assumption of contrast method is that the standardized contrasts should be independent from their estimated nodal values (Felsenstein 1985): our data were consistent with this assumption. Since branch lengths were not known for many taxa due to the composite nature of our phylogeny, we set branch length to unity.

We tested Rensch's rule separately for Odonata, Anisoptera and Zygoptera by fitting major axis regressions (MA, model II regression, Sokal & Rohlf 1981) between male (dependent variable) and female body size (independent variable) using either species-level data or phylogenetic independent contrasts (MA was forced through zero when using phylogenetic independent contrasts; Harvey and Pagel 1991; Garland et al. 1992). We use the slope of MA, since body size is usually estimated with error and other methods, such as ordinary least-squares regression assume that the measurements of independent axis are taken without an error (Sokal & Rohlf 1981). We provide the slopes of these regressions and their 95% confidence intervals (lower CI – upper CI). Slopes and confidence intervals of major axis regressions were calculated by bootstrapping the contrasts using R (R Development Core Team, http://www.R-project.org).

To test the effect of sexual selection (territoriality, male agility) on SSD we use generalised least squares (GLS; Pagel 1997, 1999; Garland & Ives 2000; Freckleton et al. 2002). GLS is a phylogenetic comparative method that incorporates the phylogenetic autocorrelation of the data in the structure of errors (variance-covariance matrix, Martins

& Hansen 1997; Freckleton et al. 2002). The structure of variance-covariance matrix was determined from the composite phylogeny of Odonata (Figure 1). GLS was used to test the maximum likelihood of the evolutionary regression coefficient between two traits (Pagel 1997, 1999). In order to improve the fit of our data to the model, we estimated the maximum likelihood value of the weighting parameter λ (see Freckleton et al. 2002), and used this λ to correct for phylogenetic effect in linear models (Pagel 1997, 1999).

Prior to analyses, we removed those taxa from the phylogeny for which no information was available on territoriality (25 species; see Appendix). We then investigated the relationship between SSD (dependent variable) and sexual selection (Territoriality, Agility; independent variables) in three separate GLS model each for Odonata, Anisoptera and Zygoptera. We included territoriality x agility interaction in the initial GLS models; however, this interaction was only significant in Odonata (see Results), so that non-significant interaction terms were eliminated from GLS models of both Anisoptera and Zygoptera (Table 1).

Finally, we tested whether the allometric relationship between body size and SSD may be explained by territoriality, male agility or their interaction using GLS models (see rationale in Szekely et al. 2004). Since Anisoptera did not exhibit this relationship (see Results), this group was not included in these analyses. In these models SSD was the dependent variable, territoriality, male agility and male size were the independent variables.

Statistical analyses were carried out in R (R Development Core Team, http://www.R-project.org). GLS calculations were carried out in R (R Development Core Team, http://www.R-project.org) using codes written by Robert P. Freckleton.

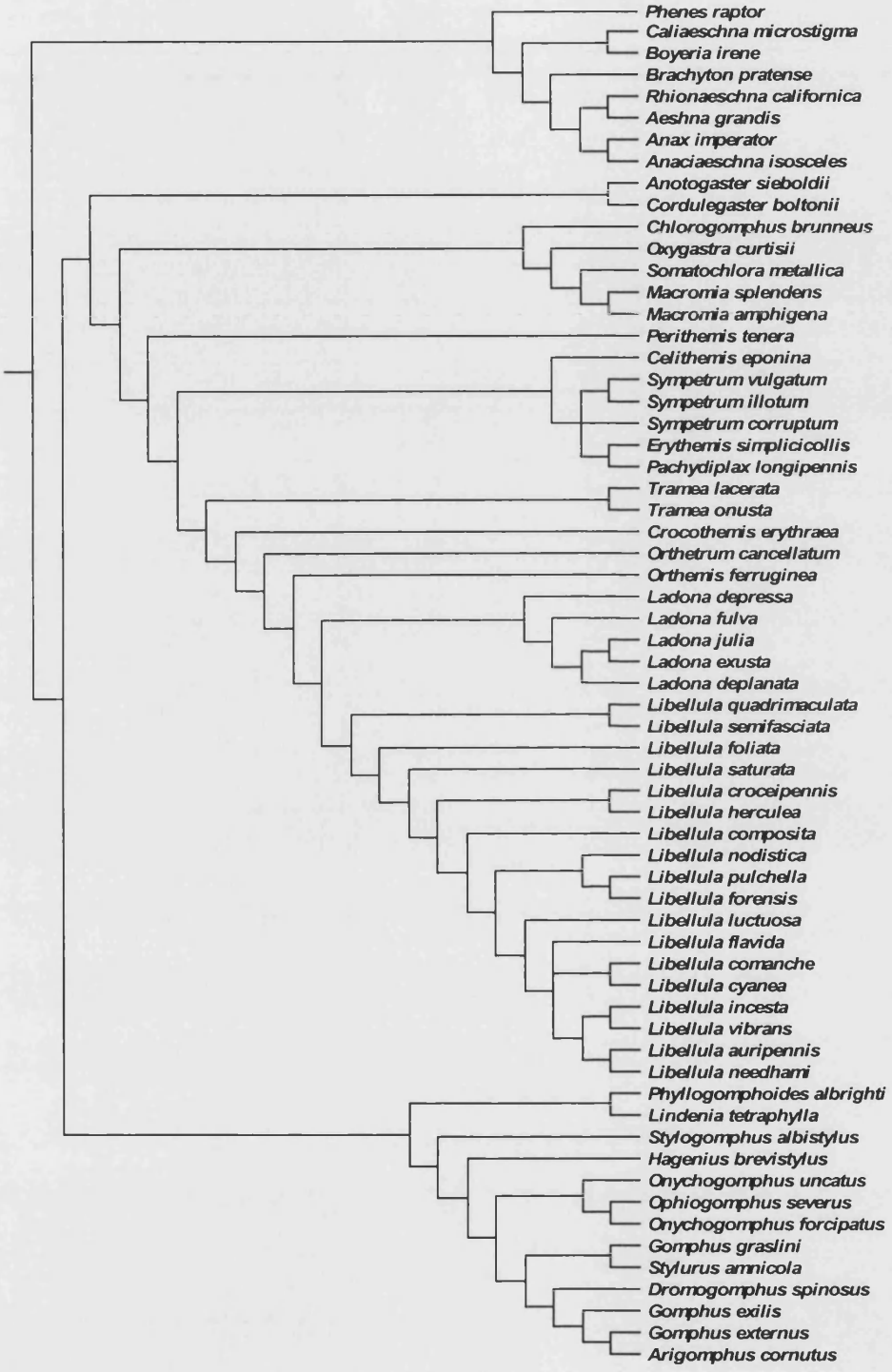

Figure 1a. Composite phylogeny of Anisoptera using Rehn's (2003) phylogram and other molecular phylogenies for families and genus (see Methods).

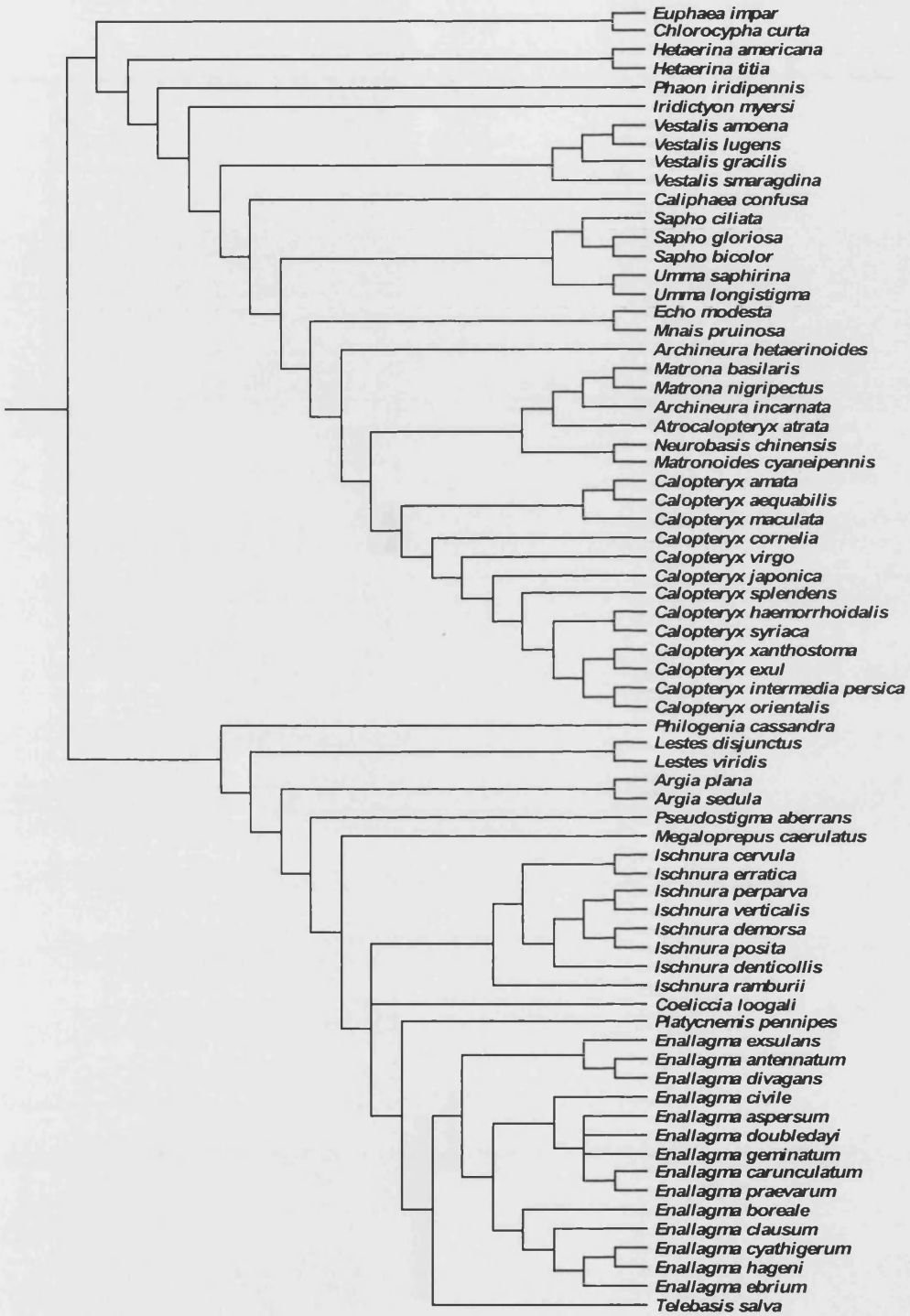

Figure 1b. Composite phylogeny of Zygoptera using Rehn's (2003) phylogram and other molecular phylogenies for families and genus (see Methods).

3. RESULTS

(a) *Distribution of SSD*

Odonates exhibit both male-biased and female-biased SSDs, although male-biased SSD is predominant (Wilcoxon one-sample test, W = 3984.5, $p = 0.0014$). In Anisoptera the median SSD is not different from 0 (W = 1159, $p = 0.303$, Figure 2), whereas most Zygoptera have male-biased SSD and their median is significantly larger than zero (W = 1808, $p = 0.001$, Figure 2).

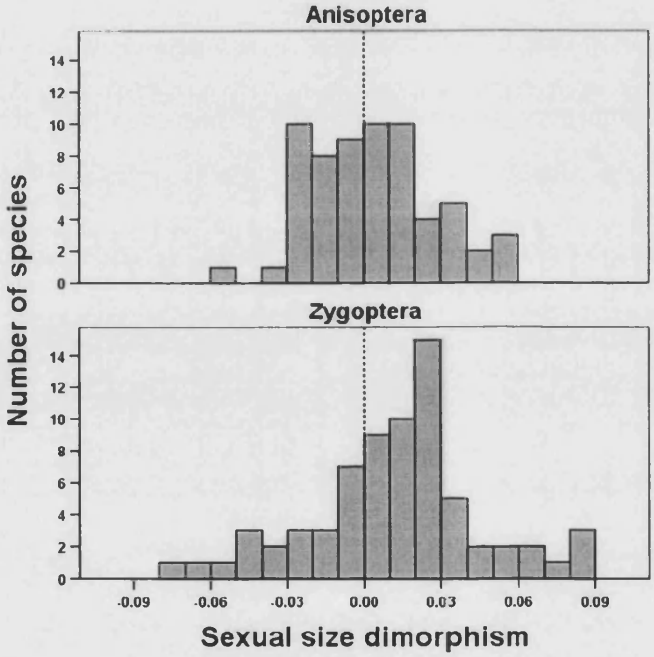

Figure 2. Distribution of sexual size dimorphism (SSD) in Anisoptera and Zygoptera. Positive SSD denote species in which males are larger than females, whereas negative SSD denote species in which females are the larger sex. The dotted lines represent monomorphism.

(b) Sexual selection

To test whether territoriality or male agility predicts SSSD, we built a series of GLS models (see Methods). First, in Odonata, there was a significant interaction between territoriality x agility ($p = 0.0013$; Model 1, Table 1; see Figure 3). Inspection of this interaction revealed that agile territorial species show a non-significant trend toward

male-biased SSD (r = -0.134, $F_{1,75}$ = 2.145, p = 0.147) whereas in non-territorial species agility is unrelated to SSD (r = 0.042, $F_{1,31}$ = 0.354, p = 0.556).

SSD in Anisoptera was not related either to territoriality or agility (p = 0.697; Model 2, Table 1), whereas territoriality was a strong predictor of SSD in Zygoptera (p = 0.0011; Model 3, Table 1). These results suggest that, overall, territoriality was a better predictor of SSD than male agility.

Table 1. Associations between SSD (dependent variable, *log*(male size) - *log*(female size)) and proxy measures of sexual selection (territoriality, male agility) using Generalised Least Squares. λ is the maximum likelihood estimate of the weighting parameter (see Freckleton et al. 2002).

Odonata

Model 1	Regression coefficient ± SE	*t*	*p*
Territoriality	0.145 ± 0.040	3.625	0.0004
Agility	0.108 ± 0.127	0.848	0.3981
Territoriality * Agility	-0.279 ± 0.084	-3.284	0.0013

Anisoptera

Model 2	Regression coefficient ± SE	*t*	*p*
Territoriality	-0.007 ± 0.009	-0.813	0.4199
Agility	-0.018 ± 0.201	-0.089	0.9290

Zygoptera

Model 3	Regression coefficient ± SE	*t*	*p*
Territoriality	0.049 ± 0.012	3.936	0.0002
Agility	-0.076 ± 0.156	-0.486	0.6287

Notes: The interaction between territoriality and agility is not significant in Anisoptera (p = 0.735) and Zygoptera (P = 0.394), therefore they were not included in the models.
Model 1: λ = 0.931. r^2 = 0.132, $F_{1,105}$ = 5.296, p = 0.0019
Model 2: λ = 0.768. r^2 = 0.014, $F_{1,52}$ = 0.362, p = 0.6974
Model 3: λ = 0.999. r^2 = 0.233, $F_{1,52}$ = 7.773, p = 0.0011

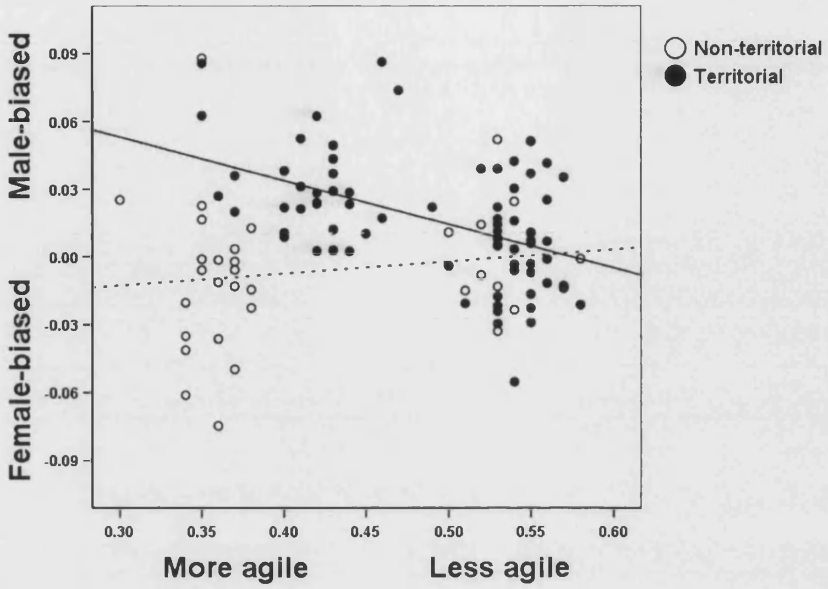

Figure 3. Sexual size dimorphism (SSD) in Odonata in relation to male agility in territorial (full dots) and non-territorial (open dots) species (see Results for explanation).

(c) Rensch's rule

Odonates exhibits Rensch's rule at species-level, since the MA slope ($\beta = 1.056$, $n = 133$ species) is greater than one (lower 95% CI – upper 95% CI: 1.020 – 1.094, $n = 133$ species). In Anisoptera the sexes are scaled isometrically ($\beta = 0.963$, 0.912 – 1.016, $n = 63$ species), whereas Zygoptera exhibit a strong allometric relationship that is consistent with the Rensch's rule ($\beta = 1.115$, 1.068 – 1.165).

These results are consistent using phylogenetic independent contrasts, since the slope of the relationship between the contrasts in male and female body size in Odonata is greater than one ($\beta = 1.627$, 1.094 – 1.254, $n = 127$ contrasts; Figure 4a). The sizes of males and females are not different from isometry in Anisoptera ($\beta = 1.118$, 0.974 – 1.223, $n = 60$ contrasts; Figure 4b), whereas Zygoptera exhibits Rensch's rule ($\beta = 1.192$, 1.116 – 1.319, $n = 66$ contrasts; Figure 4c).

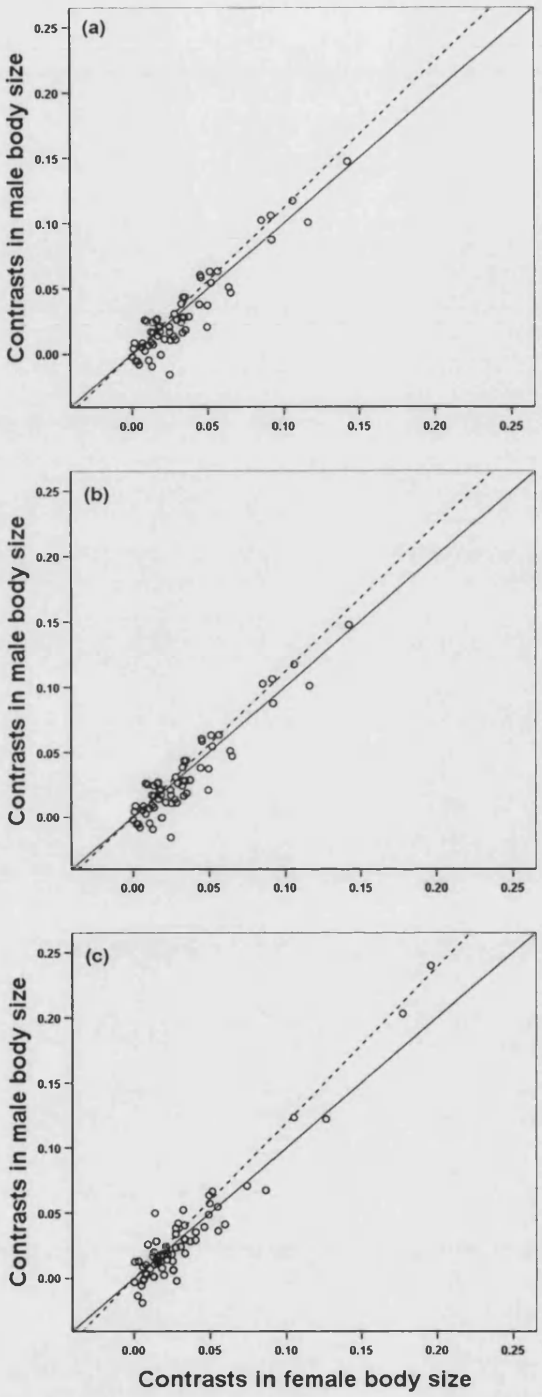

Figure 4. Phylogenetic independent contrasts of log_{10}(female size) (independent variable) and log_{10}(male size) (dependent variable) in (a) Odonata, (b) Anisoptera and (c) Zygoptera. Continuous lines indicate the isometric relationship, and dotted lines represent the fitted relationship using major axis regression through zero.

(d) Rensch's rule and sexual selection

In Odonata, both Territoriality and Agility, and their interaction contribute significantly to the allometric relationship between the sexes (Model 4, Table 2). Note however, that male size remains highly significant in all models (Models 1-5, Table 2) suggesting that the allometric relationship remains significant.

Table 2. Associations between SSD in Odonata (dependent variable), sexual selection (territoriality, agility) and male size using Generalised Least Squares (see Methods for the logic of model selection). λ is the maximum likelihood estimate of weighting parameter .

Odonata

Model	Regression coefficient ± SE	t	*p*
Model 1			
Male size	0.086 ± 0.023	3.618	0.0004
Model 2			
Territoriality	0.013 ± 0.007	1.759	0.0813
Male size	0.083 ± 0.023	3.530	0.0006
Model 3			
Agility	-0.209 ± 0.108	-1.925	0.0568
Male size	0.097 ± 0.024	4.058	0.0001
Model 4			
Territoriality	0.014 ± 0.007	1.969	0.0515
Agility	-0.225 ± 0.106	-2.117	0.0366
Male size	0.094 ± 0.023	4.020	0.0001
Model 5 (4,104)			
Territoriality	0.130 ± 0.037	3.475	0.0007
Agility	-0.021 ± 0.120	-0.175	0.8609
Territoriality * Agility	-0.250 ± 0.079	-3.137	0.0022
Male size	0.088 ± 0.022	3.904	0.0001

Model 1: $\lambda = 0.931$. $r^2 = 0.109$, $F_{1,107} = 13.096$, $p = 0.0004$
Model 2: $\lambda = 0.916$. $r^2 = 0.136$, $F_{2,106} = 8.315$, $p = 0.0004$
Model 3: $\lambda = 0.913$. $r^2 = 0.141$, $F_{2,106} = 8.673$, $p = 0.0003$
Model 4: $\lambda = 0.891$. $r^2 = 0.174$, $F_{3,105} = 7.348$, $p = 0.0001$
Model 5: $\lambda = 0.889$. $r^2 = 0.247$, $F_{4,104} = 8.454$, $p = 0.0001$
Note that comparing Akaike information content of these models would not be appropriate given missing datapoints.

In Zygoptera, territoriality but not agility (Models 2 & 3, Table 3) correlates with SSD. This is confirmed by a multivariate model that includes both variables (Model 4, Table 3). Male size, however, remains correlated with SSD in all models (Table 3).

These results suggest that sexual selection (as represented by territoriality and male agility) contribute to Rensch's rule, although it does not fully account for the allometric relationship either in Odonata or Zygoptera.

Table 3. Associations between SSD in Zygoptera (dependent variable), sexual selection (territoriality, agility) and male size using Generalised Least Squares (see Table 2 for explanations).

Zygoptera

Model	Regression coefficient ± SE	t	*p*
Model 1			
Male size	0.128 ± 0.031	4.133	0.0001
Model 2			
Territoriality	0.042 ± 0.011	3.777	0.0004
Male size	0.111 ± 0.028	3.970	0.0002
Model 3			
Agility	-0.170 ± 0.156	-1.089	0.2809
Male size	0.135 ± 0.031	4.275	0.0001
Model 4			
Territoriality	0.042 ± 0.011	3.858	0.0003
Agility	-0.190 ± 0.138	-1.371	0.1763
Male size	0.118 ± 0.028	4.195	0.0001
Model 5 (4,104)			
Territoriality	-0.046 ± 0.134	-0.345	0.7308
Agility	-0.399 ± 0.342	-1.166	0.2491
Territoriality * Agility	0.249 ± 0.372	0.667	0.5073
Male size	0.117 ± 0.028	4.115	0.0001

Model 1: $\lambda = 0.999$. $r^2 = 0.247$, $F_{1,53} = 17.083$, $p = 0.0001$
Model 2: $\lambda = 0.999$. $r^2 = 0.411$, $F_{2,52} = 17.856$, $p = 0.0001$
Model 3: $\lambda = 0.999$. $r^2 = 0.264$, $F_{2,52} = 9.166$, $p = 0.0003$
Model 4: $\lambda = 0.999$. $r^2 = 0.433$, $F_{3,51} = 12.737$, $p = 0.0001$
Model 5: $\lambda = 0.998$. $r^2 = 0.438$, $F_{4,50} = 9.584$, $p = 0.0001$

4. DISCUSSION

Odonates exhibit both male- and female-biased SSD, although males are generally larger than females across species. Similarly, males are usually larger than females across species in Zygoptera, whereas the sexes are not significantly different in size across species in Anisoptera.

What drives the observed patterns of SSD in Odonata? We investigated whether sexual selection, in the form of territoriality and male agility, was likely to drive these patterns in Odonata, Anisoptera and Zygoptera. We found that, from these estimates of sexual selection, only territoriality seems to influence SSD in Zygoptera since evolutionary increases in territoriality are correlated with evolutionary increases in male-biased SSD in this sub-order. SSD in both Odonata and Anisoptera, however, is unrelated to sexual selection. This suggests that large male body size is selectively advantageous in territorial species of Zygoptera but that it is not generally advantageous in Odonata. These results contradict the conventional prediction that male-biased SSD should be favoured in odonate species that exhibit territoriality (Anholt et al. 1991; also see Fincke et al. 1997). For example, Anholt et al. (1991) suggested that, at species level, larger-than-average male body size is advantageous for male-male competition in Odonata (Anholt et al. 1991; Andersson 1994). Indeed large male body size is advantageous in territorial species since larger males are more likely to hold or defend a territory of better quality and for longer periods (Fincke 1984; Tsubaki & Ono 1987; Serrano-Meneses et al. in press). This usually results in higher mating success for those males that defend a territory in comparison to those males that do not (Serrano-Meneses et al. in press). One possible explanation is that, during territorial contests males with high energy reserves (body fat) have an advantage over males with low reserves (Marden & Waage 1990; Plaistow & Siva–Jothy 1996; Koskimäki et al. 2004; Contreras-Garduño et al. 2006; Serrano-Meneses et al. in press) because large body size allows a greater proportion of reserves to be stored in the thoracic muscles (Serrano-Meneses et al. in press). Larger body size may benefit territorial males since they need to endure long territorial contests against conspecifics (Córdoba-Aguilar & Cordero-Rivera 2005). Thus, this may explain why male-biased SSD prevails in Zygoptera.

In Anisoptera however, the relationship between male-biased SSD and territoriality is not evident (Fincke et al. 1997). For example, satellite males of the territorial dragonfly *Libellula quadrimaculata* are generally larger than the small, active, and more successful (in terms of mating success) territorial males (Convey 1989; Andersson 1994). Other species, such as *Plathemis lydia* (Koening & Albano 1987) and *Sympetrum*

rubicundulum (Van Buskirk 1987) exhibit female-biased SSD even when males establish and defend territories. Although our results do not support this, it is possible that in Anisoptera, large male size becomes a disadvantage if males, rather than defending territories from perch sites, mostly patrol and compete for territories or females from the air. In this case, a small, more manoeuvrable size may be more beneficial to males, resulting in monomorphism or even female-biased SSD. The same principle should apply to males of non-territorial species, since they search actively for females (Corbet 1999). Nevertheless, male body size cannot evolve to be too small (relative to female size), due to energetic constraints imposed by territorial defense.

Odonata exhibit Rensch's rule both at species level and after controlling for the phylogenetic non-independence of species. This relationship, however, results from analysing both Anisoptera and Zygoptera together. Once these two groups are separated, the sizes of the sexes in Anisoptera show a scaling pattern that is not consistent with Rensch's rule. Zygoptera, interestingly, exhibits the full scope of Rensch's rule: the extent of SSD increases with body size in species in which the males is the larger sex is much, whereas it decreases with body size in species where females are larger than males (Abouheif & Fairbairn 1997; Fairbairn 1997). Although the proposed explanations for Rensch's rule have been numerous (i.e. genetic correlation between the sexes, energy optimisation, differential niche-utilisation; Fairbairn 1997), only sexual selection has been shown to drive the observed pattern (i.e. shorebirds; Székely et al. 2004). We investigated whether sexual selection drives Rensch's rule in Odonata and Zygoptera, and found that sexual selection undoubtedly influences the allometric relationship between the sexes. However, our results indicate that sexual selection is not the only explanation for Rensch's rule. Therefore other ecological variables (i.e. differential resource-utilisation, difference in habitats between the sexes and fertility selection acting on females) may contribute to the observed allometric pattern.

Or study can be complemented in various ways. First, more detailed descriptions of odonate mating systems are needed to gain a better understanding of intrasexual competition. Second, there may exist other morphological variables that are better

estimates of male agility, however, this would require data on body mass (Grabow & Rüppell 1995; Wakeling 1997), which is extremely difficult to obtain for odonates. Third, the incorporation of other ecological variables, such as habitat type and diet would broaden our understanding of the difference in body size between the sexes. This would however require the description of these variables, which are difficult to find in the literature. Finally, female fecundity should also be incorporated, since female size may be under the influence of fecundity selection, and thus may contribute to the patterns of SSD.

In conclusion, our study reveals that sexual selection drives male-biased SSD in Zygoptera, however, this relationship is not ubiquitous in Odonata and Anisoptera and future studies should aim to achieve a better understanding of other evolutionary processes that may influence SSD in Odonata.

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APPENDIX

Data used in the study and sources. Male and female body size is the mean body length of at least 3 individuals per sex. T, territorial; NT, non-territorial. Agility is the proportion of the proximal area of the wing; low proportions denote more agility whereas high proportions denote wings for faster flight. (1) Azpilicueta-Amorín, M. unpubl.; (2) Brooks & Lewington 2004; (3) Corbet 1999; (4) Cordero 1988; (5) Córdoba-Aguilar 2002; (6) Córdoba-Aguilar, A. pers. obs.; (7) D'Aguilar & Dommanget 1998; (8) Dunkle 1989; (9) Dunkle 2000; (10) Fincke 1987; (11) Fincke, O. M. pers. comm.; (12) González-Soriano, E. pers. collection; (13) González-Soriano, E. pers. comm.; (14) Hamalainen & Pinratana 1999; (15) Johansson et al. 2005; (16) Kumar & Prasad 1977; (17) Logan 1967; (18) Manolis 2003; (19) McVey 1988; (20) Natural History Museum, London, Odonata collection; (21) Novelo-Gutiérrez, R. pers. comm.; (22) Ocharán 1987; (23) Orr 2003; (24) Serrano-Meneses et al. in press; (25) Silsby & Parr 2001; (26) Siva-Jothy et al. 1998; (27) Sokolovska et al. 2000; (28) Tsubaki et al. 1997; (29) Tynkkynen, K. pers. comm.; (30) Walker 1958; (31) Watanabe et al. 1998.

Species	Male size in cm	Female size in cm	Mating system	Agility	References
Aeshna grandis	7.039	6.857	T	0.53	15, 20
Anaciaeschna isosceles	6.417	6.745	T	0.53	2, 20
Anax imperator	6.927	7.194	T	0.55	1, 4
Anotogaster sieboldii	8.976	9.596	T	0.55	20, 28
Archineura incarnata	8.013	7.617	T	0.49	3, 20
Archineura hetaerinoides	7.556	7.964		0.48	20
Argia plana	3.420	3.510	NT	0.36	3, 20
Argia sedula	3.320	3.120	T	0.36	3, 20
Arigomphus cornutus	4.845	4.757		0.52	20
Atrocalopteryx atrata	6.294	5.985	T	0.40	3, 20
Boyeria irene	6.309	6.153	NT	0.50	22
Brachyton pratense	5.470	5.520	T	0.50	2, 22

Caliaeschna microstigma	6.298	6.714		0.52	20
Caliphaea confusa	4.462	4.228		0.36	20
Calopteryx aequabilis	5.092	4.677	T	0.43	18, 20
Calopteryx amata	5.537	5.238	T	0.42	3, 20
Calopteryx cornelia	7.135	6.968	T	0.45	3, 20
Calopteryx exul	5.184	4.983	T	0.46	7, 20
Calopteryx haemorrhoidalis	4.980	4.717	T	0.42	5, 20
Calopteryx intermedia persica	4.659	4.544		0.41	20
Calopteryx japonica	5.749	5.710	T	0.43	20, 31
Calopteryx maculata	5.204	4.766	T	0.40	20, 27
Calopteryx orientalis	4.599	4.548		0.41	20
Calopteryx splendens	4.557	4.339	T	0.41	20, 29
Calopteryx syriaca	5.049	4.898		0.40	20
Calopteryx virgo	4.679	4.431	T	0.42	20, 29
Calopteryx xanthostoma	4.640	4.527	T	0.40	6, 20
Celithemis eponina	3.838	3.627	NT	0.54	3, 20
Chlorocypha curta	3.034	2.627	T	0.35	3, 20
Chlorogomphus brunneus	8.150	7.805		0.55	20
Coeliccia loogali	4.913	4.823		0.33	20
Cordulegaster boltonii	6.855	7.785	T	0.54	3, 22
Crocothemis erythraea	3.898	3.536	T	0.54	1, 2
Dromogomphus spinosus	5.732	6.183	NT	0.53	20, 30
Echo modesta	5.682	5.323	T	0.42	3, 20
Enallagma antennatum	2.721	3.132	NT	0.34	20, 30
Enallagma aspersum	2.808	3.148	NT	0.37	20, 30
Enallagma boreale	3.100	3.265	NT	0.38	20, 27
Enallagma carunculatum	3.229	3.244	NT	0.37	18, 20
Enallagma civile	3.210	3.065	T	0.37	3, 12
Enallagma clausum	3.198	3.295	NT	0.37	18, 20
Enallagma cyathigerum	3.035	3.075	NT	0.37	12, 18
Enallagma divagans	2.890	3.174		0.37	20
Enallagma doubledayi	2.916	3.281		0.37	20
Enallagma ebrium	3.001	2.914	NT	0.38	20, 27
Enallagma exsulans	3.245	3.400	NT	0.34	12, 30

Enallagma geminatum	2.540	2.570		0.38	20
Enallagma hageni	2.807	2.902	NT	0.38	20, 27
Enallagma praevarum	3.075	3.050	NT	0.37	18, 20
Erythemis simplicicollis	4.350	4.410	T	0.54	12, 19
Euphaea impar	5.323	4.707		0.40	20
Gomphus exilis	4.142	4.008	NT	0.52	20, 30
Gomphus externus	5.236	5.334	NT	0.52	18, 20
Gomphus graslini	4.662	4.805	NT	0.53	18, 20
Hagenius brevistylus	8.116	8.210		0.55	20
Hetaerina americana	4.113	3.470	T	0.47	24
Hetaerina titia	4.801	3.935	T	0.46	6, 20
Iridictyon myersi	6.578	5.871	T	0.43	20, 25
Ischnura cervula	2.651	2.915	NT	0.34	18, 20
Ischnura demorsa	2.545	2.505		0.35	12
Ischnura denticollis	2.745	2.590	NT	0.30	12, 18
Ischnura erratica	3.372	3.382	NT	0.36	18, 20
Ischnura perparva	2.646	2.652	NT	0.35	18, 20
Ischnura posita	2.400	2.310	NT	0.35	6, 12
Ischnura ramburii	2.805	3.330	NT	0.36	8, 12
Ischnura verticalis	2.677	2.902	NT	0.34	10, 20
Ladona deplanata	3.290	3.209	T	0.55	9, 20
Ladona depressa	4.589	4.479	T	0.55	3, 20
Ladona exusta	3.355	3.379	T	0.55	8, 20
Ladona fulva	4.320	4.029	T	0.54	2, 20
Ladona julia	4.004	3.927	T	0.53	18, 20
Lestes disjunctus	3.563	3.873	NT	0.36	20, 27
Lestes viridis	4.484	4.127	T	0.37	1, 4
Libellula auripennis	5.210	5.001		0.54	20
Libellula comanche	4.907	4.946	T	0.54	12, 18, 20
Libellula composita	4.410	4.032	T	0.52	18, 20
Libellula croceipennis	5.490	4.880	T	0.55	12, 18
Libellula cyanea	4.300	4.161	T	0.53	9, 20
Libellula flavida	4.478	4.663	T	0.53	9, 20
Libellula foliata	4.566	4.576	T	0.56	13, 20

Libellula forensis	4.648	4.224	T	0.56	12, 18
Libellula herculea	5.110	5.470	T	0.53	12, 13
Libellula incesta	5.088	5.027	T	0.53	9, 20
Libellula luctuosa	4.555	4.690	T	0.57	12, 27
Libellula needhami	5.394	5.333	T	0.53	9, 12, 20
Libellula nodistica	4.740	4.560	T	0.53	12, 17
Libellula pulchella	5.061	4.970	T	0.53	18, 20
Libellula quadrimaculata	4.207	4.275	T	0.55	1, 15
Libellula saturata	5.480	5.170	T	0.56	12, 13
Libellula semifasciata	4.305	4.230		0.54	20
Libellula vibrans	5.692	5.838		0.56	20
Lindenia tetraphylla	7.000	6.400	T	0.53	3, 22
Macromia amphigena	7.300	7.185	T	0.56	3, 20
Macromia splendens	6.498	6.869	T	0.53	1
Matrona basilaris	6.620	6.580	T	0.42	20, 26
Matrona nigripectus	6.394	6.265	T	0.40	14, 20
Matronoides cyaneipennis	6.424	5.910		0.45	20
Megaloprepus caerulatus	12.000	9.850	T	0.35	3, 11, 20
Mnais pruinosa	5.651	5.008	T	0.41	3, 20
Neurobasis chinensis	5.700	5.668	T	0.44	16, 20
Onychogomphus forcipatus	5.052	4.482	NT	0.53	22
Onychogomphus uncatus	5.187	4.931	T	0.53	15, 22
Ophiogomphus severus	4.844	4.762	T	0.55	9, 20
Orthemis ferruginea	5.060	4.665	T	0.57	12, 18
Orthetrum cancellatum	4.693	4.629	T	0.55	1, 15
Oxygastra curtisii	4.257	4.485	T	0.55	1, 2
Pachydiplax longipennis	3.953	3.512	T	0.55	18, 20
Perithemis tenera	2.270	2.380	T	0.51	12, 18
Phaon iridipennis	6.861	6.415	T	0.43	3, 20
Phenes raptor	8.536	8.109		0.55	20
Philogenia cassandra	4.741	4.744		0.34	20
Phyllogomphoides albrighti	6.205	6.157	T	0.54	9, 20
Platycnemis pennipes	3.766	3.574	NT	0.35	2, 20
Pseudostigma aberrans	13.900	11.350	NT	0.35	11, 20, 21

Rhionaeschna californica	5.564	5.110	T	0.55	12, 18
Sapho bicolor	6.059	5.638	T	0.41	20, 25
Sapho ciliata	6.025	5.642	T	0.44	20, 25
Sapho gloriosa	6.941	6.578	T	0.44	20, 25
Somatochlora metallica	4.974	5.250	NT	0.54	2, 20
Stylogomphus albistylus	3.681	3.918		0.52	20
Stylurus amnicola	4.780	4.947	NT	0.51	9, 20
Sympetrum corruptum	4.007	4.137	T	0.57	9, 20
Sympetrum illotum	3.59	3.687	T	0.56	18, 20
Sympetrum vulgatum	3.371	3.249	T	0.54	2, 3, 22, 15
Telebasis salva	2.513	2.547	NT	0.35	18, 20
Tramea lacerata	4.903	4.912	NT	0.58	12, 18
Tramea onusta	4.347	4.564	T	0.58	9, 20
Umma longistigma	5.666	5.127	T	0.43	20, 25
Umma saphirina	5.329	5.182	T	0.43	20, 25
Vestalis amoena	5.616	4.865	T	0.42	20, 23
Vestalis gracilis	6.249	6.062		0.41	20
Vestalis lugens	5.155	4.988		0.40	20
Vestalis smaragdina	5.300	5.203		0.39	20

Chapter 6. *Sexual size dimorphism in seabirds: sexual selection, fecundity selection and differential niche-utilisation*

M. Alejandro Serrano-Meneses & Tamás Székely (2006)

Oikos 113, 385-864

Details and extent of the contributions by authors

M. A. Serrano-Meneses: data collection, phylogenetic analyses, manuscript writing (50%)

T. Székely: suggestions to improve the study, general editing of the manuscript (50%)

OIKOS 113: 385–394, 2006

Sexual size dimorphism in seabirds: sexual selection, fecundity selection and differential niche-utilisation

Martín-Alejandro Serrano-Meneses and Tamás Székely

Serrano-Meneses, M. A. and Székely, T. 2006. Sexual size dimorphism in seabirds: sexual selection, fecundity selection and differential niche-utilisation. – Oikos 113: 385–394.

Seabirds exhibit a range of sexual size dimorphism (SSD) that includes both male-biased (males > females) and female-biased SSD (males < females). Here we use phylogenetic comparative methods to test the selective processes that may influence their SSD. Using phylogenetically independent contrasts we show that the sizes of males and females are scaled isometrically in seabirds. We also test three functional hypotheses of SSD: sexual selection, fecundity selection and differential niche-utilisation. First, we found support for the sexual selection hypothesis, even though seabirds are socially monogamous and, as a consequence one might expect sexual selection to be weak. We show that SSD is correlated with an aspect of sexual selection, the agility of male displays, since in species that exhibit aerial displays the males are smaller (relative to the female) than in species in which the males display on the ground. Second, our results are not consistent with the fecundity selection hypothesis, since contrary to the predicted trend, female seabirds lay larger eggs in male-biased species than in female-biased ones. Finally, our results are not consistent with a previous study of the differential niche-utilisation hypothesis, since we found no relationship between SSD and ocean primary productivity in the breeding areas. Taken together, we suggest that seabird SSD is most consistent with the sexual selection hypothesis via the agility of male displays. Nevertheless, further data and tests are required to establish whether different resource utilisation by males and females may also select for SSD.

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A difference in body size between males and females, sexual size dimorphism (SSD), is a prevailing characteristic in a wide range of animal taxa (Andersson 1994, Fairbairn 1997, Blanckenhorn 2000). Three major processes have been proposed that produce and maintain SSD (reviewed by Jehl and Murray 1986, Hedrick and Temeles 1989, Andersson 1994). First, Darwin (1871) noted that if sexual selection is more intense in one sex than in the other (either via male–male competition or female choice) then SSD is likely to emerge (Payne 1984, Webster 1992, Owens and Hartley 1998). The direction of sexual selection on body size, however, may depend on whether the competition occurs on the ground or in the air (Payne 1984, Jehl and Murray 1986, Figuerola 1999, Székely et al. 2004). For instance, sexual selection is expected to favour small body size in males that exhibit aerial displays (Andersson and Norberg 1981, Hakkarainen et al. 1996, Blomqvist et al. 1997, Székely et al. 2000), whereas if males display or fight on the ground, then large size is often advantageous (Clutton-Brock et al. 1982, Anderson and Fedak 1985, Lindenfors and Tullberg 1998, Székely et al. 2000, Lindenfors et al. 2002).

Second, fecundity selection for large size has been proposed to explain female-biased SSD in many invertebrates, fish and birds (Andersson 1994). The optimal

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body size may differ between the sexes if large females have higher reproductive success due to their higher capacity for laying eggs (Ridley and Thompson 1979, Wiewandt 1982, Honěk 1993), or if males prefer large females to small ones (Sandercock 1998, 2001). Consistently, studies of spiders, insects and ectotherm vertebrates, such as fishes and frogs, have demonstrated positive relationships between female body size and fecundity (Shine 1979, Head 1995, Prenter et al. 1999).

Third, males and females may have different body sizes to avoid resource–competition, or enhance feeding efficiency (differential niche-utilisation, Selander 1966, Shine 1989, Thom et al. 2004). Since body size or morphology is often evolved to adapt to different niches (Shine 1989, Sandercock 2001), sexually dimorphic pairs can exploit a wider range of resources than monomorphic ones (Figuerola 1999).

A puzzling allometric relationship exists between body size and sexual size dimorphism in many animals: in taxa with male-biased dimorphism SSD increases with body size, whereas the extent of female-biased SSD decreases with body size (Rensch's rule, Abouheif and Fairbairn 1997, Fairbairn 1997). Several hypotheses have been suggested to explain Rensch's rule, and a recent comparative study showed that the Rensch's rule was most consistent with selection pressures emerging from two aspects of sexual selection: intensity of male–male competition and agility of male displays (Székely et al. 2004).

Seabirds (bird taxa living in and making their living from marine environments; 193 species, Monroe and Sibley 1993, Diomedeinae, Fregatidae, Hydrobatinae, Pelecaninae, Phaethontidae, Phalacrocoracidae, Procellariinae, Spheniscidae and Sulidae, excluding the superfamilies Ardeoidea, Ciconiodea, Phoenicopteroidea, Scopoidea, Threskiornithoidea the family Anhingidae and the sub-family Balaenicipitinae) are excellent organisms to investigate functional hypotheses of SSD, since they exhibit both male-biased and female-biased dimorphisms. A previous study found that seabirds in more productive areas exhibited male-biased SSD (Fairbairn and Shine 1993), and showed that seabirds exhibited SSD consistent with Rensch's rule. Fairbairn and Shine (1993) however, only used Southern Hemisphere species, and investigated the putative explanatory variables mostly in isolation from each other. Furthermore, Fairbairn and Shine (1993) did not investigate the influence of sexual selection on SSD, since all seabirds are socially monogamous. Nevertheless, sexual selection may still influence SSD in monogamous species via selecting for agility (and thus small size) in males (Jehl and Murray 1986, Figuerola 1999, Székely et al. 2000).

Here we first test whether seabirds exhibit Rensch's rule using species from both Northern and Southern Hemispheres. Second, we test three functional hypotheses of SSD: (i) if sexual selection influences SSD, we expected to find a relationship between agility of male displays and SSD: male-biased SSD should occur with non-agile displays. (ii) If fecundity selection influences female size, then both egg size and clutch size should increase with the size of female relative to male. (iii) If differential niche-utilisation of resources influences SSD, following Fairbairn and Shine (1993) we predict that differences between female and male size should be more pronounced in those species that breed in areas of low ocean primary productivity, because the male and the female of a breeding pair should specialise in exploiting different resources to avoid food competition. Conversely, those species that have highly productive feeding areas around their breeding site should be monomorphic.

Methods

Data

We collected data on body mass, clutch size and egg size, and descriptions of male display behaviour from published sources (handbooks and reference books; Appendix 1). We used those seabird taxa for which body mass data were available. We also recorded the geographic location of the breeding site where the morphometric measures were taken to derive the ocean primary productivity. Male displays were scored blindly to the identity of species by three observers using the descriptions of male displays as follows: (1) ground display; (2) both ground and aerial displays: males displayed from nests or territories but also engaged in aerial activities such as the stealing of nest material from other nests; (3) aerial display: males displayed acrobatically or non-acrobatically in the air (see similar approach by Figuerola 1999 and Székely et al. 2000; Appendix 1). The correlations between the scores of three observers were high (all Spearman's rank correlation coefficients $r_s > 0.883$, $p = 0.001$, $n = 71$).

Ocean productivity was derived from a composite map of September 1998 – August 1999 (SEAWIFS: estimated primary productivity map, http://marine.rutgers.edu/opp/swf/Production/results/all2_swf.html). Ocean primary productivity was estimated from visible light reflectance using the consensus algorithm for chlorophyll concentration (Behrenfeld and Falkowski 1997). We calibrated this map such that the value of each pixel (every pixel measures approximately 18 km per side, i.e. 324 km^2) corresponded to the chlorophyll concentration (grams of carbon m^{-2}) by plotting the palette order of pixels in the colour-scale of the map against chlorophyll concentration to obtain the relationship OP = 0.5556/palette order. This was applied as an image calculation using the image software Idrisi 3.32 (Clark Labs: http://www.clarklabs.org). At each breeding location, ocean productivity was measured in 16 surrounding pixels

(within an estimated area of 5184 km^2). For those breeding locations where information was not available (i.e. Antarctica), we estimated the OP at the closest breeding location. The average ocean production of 16 pixels was used in the analyses, with a minimum value of productivity of 56 and a maximum of 450 g of carbon m^{-2}.

Phylogeny

We used a composite phylogeny of seabirds (5 families, 4 sub-families, 73 species; Fig. 1) by augmenting the DNA–DNA hybridisation phylogeny of Sibley and Ahlquist (1990; Fig. 366–368) with recent molecular phylogenies. Where possible, we included changes in the existing phylogeny if supported by new molecular evidence. Thus, Diomedeinae, Procellariinae, Hydrobatinae and Spheniscidae were taken from Nunn and Stanley (1998), Fregatidae was taken from Kennedy and Spencer (2004), Pelecaninae was taken from Sibley and Ahlquist (1990; Fig. 367), Sulidae was taken from Friesen and Anderson (1997) and Phalacrocoracidae was taken from on Kennedy et al. (2000). To see whether our results were sensitive to the phylogenetic hypothesis, we

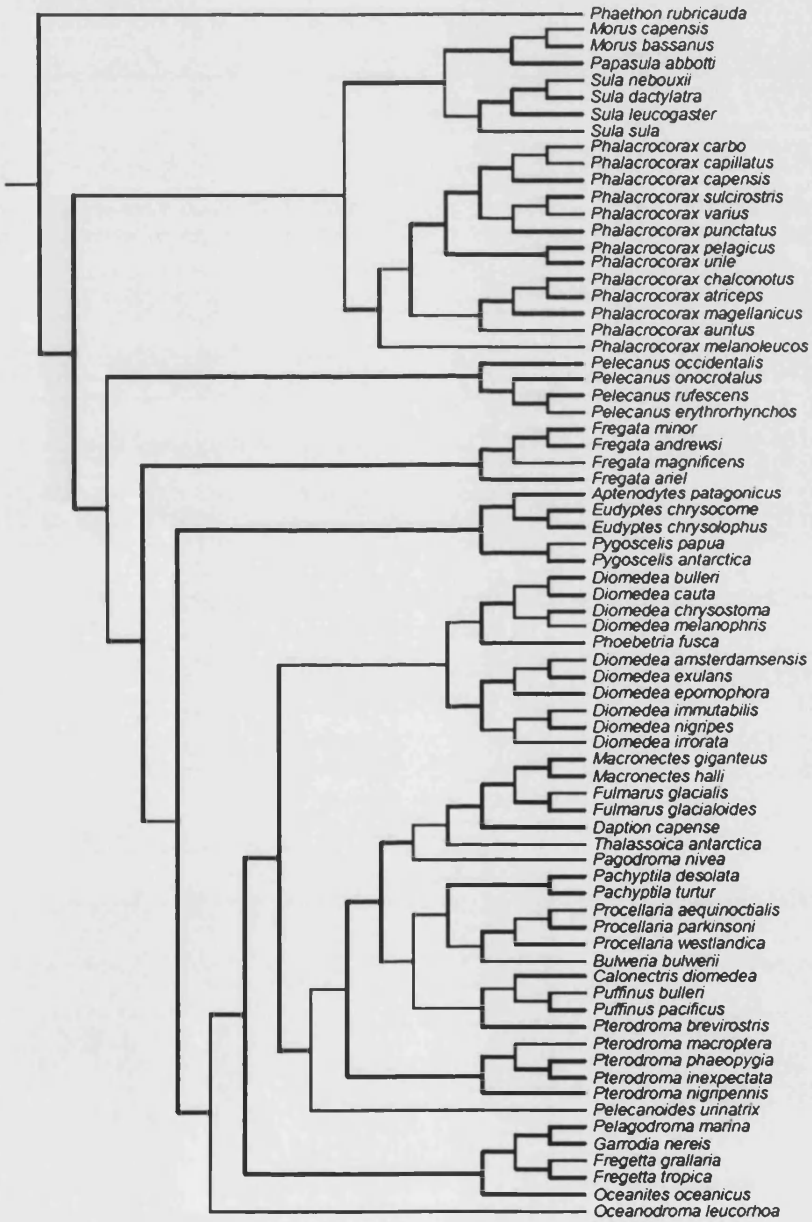

Fig. 1. Composite seabird phylogeny composed of Sibley and Ahlquist's (1990) UPGMA tree with molecular phylogenies of individual seabird families.

reanalysed our data using Kennedy and Page (2002) seabird supertree. Since Kennedy and Page (2002) do not include *Pterodroma brevirostris* in their supertree, this species was not included in our re-analyses.

Phylogenetic analyses with Kennedy and Page (2002) were largely consistent with our main results; here we only provide those that are qualitatively different.

Phylogenetic analyses

We used the phylogenetic independent contrasts method of Felsenstein (1985) as implemented by CAIC (Purvis and Rambaut 1995) to control for phylogenetic non-independence of species (Harvey and Pagel 1991). An assumption of Felsenstein's method (1985) is that the standardized contrasts should be independent from their estimated nodal values: the distributions of our data were consistent with this assumption. All branch lengths were set to equal values.

We tested Rensch's rule by fitting a major axis regression (model II regression, Sokal and Rohlf 1981) through the origin using phylogenetic independent contrasts (Harvey and Pagel 1991, Garland et al. 1992). We provide the slopes of these regressions and their 95% confidence intervals (lower CI – upper CI). Slopes and confidence intervals of major axis regressions were calculated by bootstrapping the contrasts using R (R Development Core Team, http://www.R-project.org).

Data were $\log_{10}$ transformed prior to the analyses, except display scores. SSD was calculated as contrasts in $\log_{10}$ (male body mass) – contrasts in $\log_{10}$ (female body mass). Log transformed data are commonly used to calculate SSD. The difference between male and female size expressed as a logarithm is more accurate than ratios because ratios have undesired statistical properties (Smith 1999). Egg size (in mm^3) was estimated by calculating the egg-index (egg-length $\times$ breadth^2).

We tested functional hypotheses of SSD by investigating the relationship between SSD (dependent variable) and explanatory variables (display behaviour, clutch size and egg size, and ocean productivity) in bivariate and multivariate least squares regressions. These regressions were forced through the origin (Harvey and Pagel 1991), and when appropriate, we provide partial correlation coefficients. We also tested whether absolute SSD was related to ocean productivity since the differential niche-utilisation hypothesis does not make a priori assumption on the direction of SSD. Therefore, we performed a bivariate regression using the absolute values of contrasts in SSD (dependent variable) and contrasts in ocean productivity (independent variable). We constructed a multivariate model that initially included all four hypothesised explanatory variables (display behaviour, clutch size, egg size, ocean productivity), and then used backward elimination to remove non-significant variable(s). Then the influences of all pairwise statistical interactions of the initial model were tested on SSD, however, only the effect of clutch size $\times$ egg size was marginally significant ($p = 0.068$). Since clutch size and egg size had similar effects on SSD in regards to sign and magnitude, this interaction was not considered further.

Statistical calculations were carried out using SPSS (Ver. 11) and Minitab (release 12).

Results

Distribution of SSD among seabird families

Seabirds exhibit both male- and female-biased SSDs, and the median SSD is significantly different among families (Fig. 2, Kruskal–Wallis $H = 46.482$, $df = 8$, $p = 0.0001$). Males are larger than females in Diomedeinae (Wilcoxon one-sample tests, $W = 66$, $p = 0.004$, $n = 11$), Phalacrocoracidae ($W = 91$, $p = 0.002$, $n = 13$), Procellariinae ($W = 233$, $p = 0.001$, $n = 22$), Pelecaninae ($W = 10$, $p = 0.1$, $n = 4$) and Spheniscidae ($W = 13$, $p = 0.178$, $n = 5$) although the trend in the latter two groups is not supported statistically. Also, there is a strong, but statistically non-significant female-biased SSD in Sulidae ($W = 2$, $p = 0.052$, $n = 7$), Fregatidae ($W = 0$, $p = 0.1$, $n = 4$), and Hydrobatinae ($W = 0$, $p = 0.059$, $n = 6$). SSD in Phaethontidae ($W = 0$, $p = 1$, $n = 1$) is not different from unity.

Rensch's rule

At species level, seabirds exhibit the Rensch's rule since the slope of major axis regression ($b = 1.037$, $n = 73$ species) is greater than one (lower 95% CI – upper 95% CI: 1.015–1.060, $n = 73$ species). However, the results of

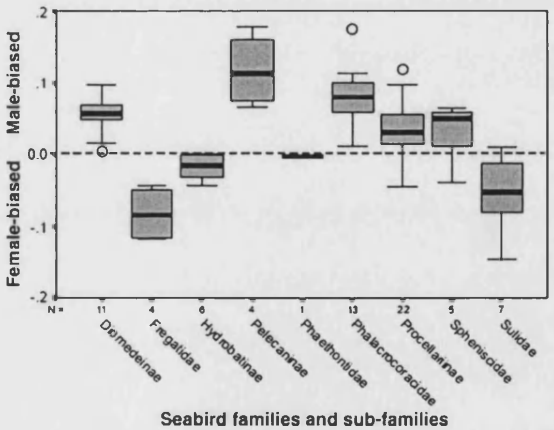

Fig. 2. Sexual size dimorphism in body mass of seabird families and sub-families (median, lower and upper quartiles; whiskers are extremes). N denotes the number of species in each family (or sub-family), and the dotted line represents monomorphism.

major axis regression using phylogenetically independent contrasts is not consistent with the species-level result, since the confidence intervals include the isometric relationship (b = 1.044, 0.929–1.067, n = 72 contrasts; Fig. 3). The latter result remains consistent when one outlier data point was excluded from the analysis (data point 'A' in Fig. 3, b = 1.054, 0.999–1.088, n = 71 contrasts). The data point was excluded under the rationale that one single outlier may bias the results. However, using the alternative supertree of Kennedy and Page (2002), we found weak support for Rensch's rule (b = 1.056, 1.010–1.079, n = 68 contrasts).

Sexual selection, fecundity selection and differential niche-utilisation

Evolutionary increases in male display agility correlated with evolutionary changes toward female-biased SSD (r = −0.233, p = 0.050, n = 70 contrasts, Fig. 4). Since display contrasts were highly variable around zero, we repeated the latter analysis by excluding them from the bivariate regression (see similar approach by Owens and Hartley 1998). The relationship between SSD and display remained significant (r = −0.325, p = 0.030, n = 44 contrasts). Furthermore, by excluding an outlier (data point 'B' in Fig. 4) the relationship became stronger (r = −0.338, p = 0.025, n = 43)

Both measures of fecundity were related to SSD (r^2 = 0.129, p = 0.011, n = 66 contrasts; clutch size, partial r = 0.253, p = 0.035; egg size, partial r = 0.306, p = 0.010). Thus, evolutionary increases toward large males relative to females were associated with increases in both clutch size and egg size. Note that these relationships are the opposite of the predicted ones:

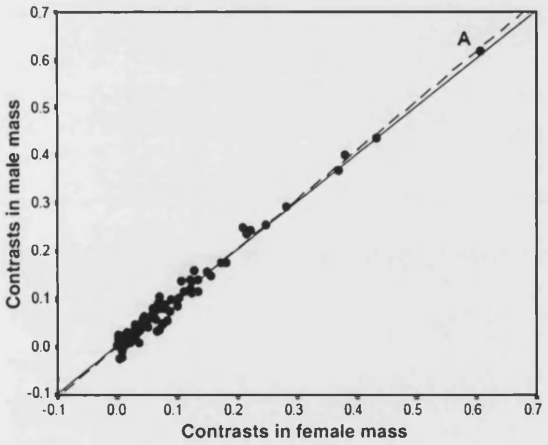

Fig. 3. The relationship between independent contrasts of $\log_{10}$(male body mass) and $\log_{10}$(female body mass). The continuous line indicates the isometric relationship, and the dotted line represents the fitted relationship using major axis regression through zero (b = 1.044, n = 72 contrasts).

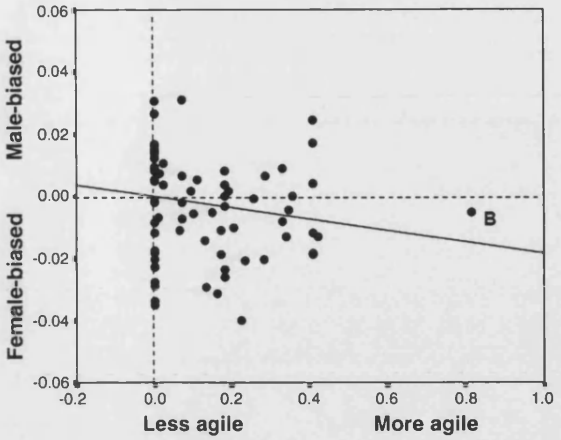

Fig. 4. Phylogenetically independent contrasts in sexual size dimorphism (SSD) and male display agility (r = −0.233, p = 0.050, n = 70 contrasts). Regression was forced through the origin.

since increases in clutch and egg size were expected to increase with large female size relative to males.

Ocean productivity was not related to SSD, since there was no relationship between SSD and the ocean productivity around the breeding areas (r = 0.0001, p = 0.903, n = 72 contrasts). Similarly, when we tested for the relationship between absolute values of SSD and ocean productivity (r = 0.004, p = 0.975, n = 72), no relationship was found.

These results are consistent with the full multivariate model, since ocean productivity was not retained in the final model, whereas egg size remained correlated with SSD, and clutch size and display behaviour were marginally significant (Table 1). Note, that the direction of relationships between SSD, clutch size and egg size remained inconsistent with the fecundity hypothesis.

Discussion

Our study provided four major results. First, at species level, seabirds appear to follow Rensch's rule, however, this relationship no longer holds when we use the phylogenetically independent contrasts using composite phylogeny. Note, however, that once an outlier value was excluded from the analyses, the results were near to significance. Also, when we use Kennedy and Page (2002) seabird supertree we find weak support for Rensch's rule. Thus, seabirds, unlike shorebirds, hummingbirds and bustards (Colwell 2000, Raihani et al. 2006), Rensch's rule does only exist as a trend but we conclude that it is not statistically significant. In shorebirds, Székely et al. (2004) proposed that the intensity of sexual selection and the agility of male displays influenced the evolution (or maintenance) of Rensch's rule. Their findings are consistent with our work, since

Table 1. Final multivariate model using phylogenetic independent contrasts. The dependent variable is contrasts in $\log_{10}$(male body mass) – contrasts in $\log_{10}$(female body mass) ($r^2 = 0.170$, $n = 69$ contrasts).

Independent variables	Slope ± SE	Partial correlation coefficient	p
Clutch size	0.09718 ± 0.050	0.235	0.054
Egg size	0.05200 ± 0.020	0.300	0.013
Display behaviour	−0.01541 ± 0.009	−0.214	0.080

seabirds are socially monogamous whereas shorebirds also exhibit social polygamy; therefore sexual selection in seabirds appears to be less intense than in shorebirds. Note that various other hypotheses outlined by Fairbairn (1997) remained untested by both Székely et al. (2004) and us.

Second, male agility correlated with SSD. Thus sexual selection, in the form of male display behaviour, appears to influence SSD in seabirds. In this work we use a paraphyletic group (seabirds), so one potential criticism is that the inclusion or exclusion of related taxa might change our results. However, we believe that this is unlikely because we do not use ancestral states to perform directional analyses as we are interested in the phylogenetic independence of the taxa. Males tend to be larger, relative to females in those species where males display on the ground, whereas selection appears to favour small male size in those species that exhibit aerial display. The effect of male display behaviour was moderate, although we should bear in mind that agility, as represented by the scores, was a crude variable. Nevertheless, strong ecological constraints (viability selection, Blanckenhorn 2000) and low levels of mating competition (all seabirds are socially monogamous) may restrain the sexes to similar sizes. Note, that correlational methods, such as phylogenetic independent contrasts, cannot separate cause and effect. Further comparative works using directional phylogenetic methods are thus needed (Pagel 1994).

Third, both clutch size and egg size correlate with SSD, however, these relationships are not consistent with the fecundity selection hypothesis. It is not obvious why SSD increases with egg size and clutch-size. One potential explanation is that males of all seabirds contribute to incubation and brood-rearing, so that increasing male size allows efficient incubation. On the other hand, it is possible that strong genetic correlation between male and female size may also have an indirect effect on egg and clutch size.

Fourth, unlike Fairbairn and Shine's (1993) study, ocean productivity was unrelated to SSD. The lack of relationship between their results and ours may be due to differences in ocean productivity data: Fairbairn and Shine estimated minimum levels for rates of primary production of the surrounding ocean in the breeding areas (rate of carbon fixation in g m^{-2}, to the nearest 50 g) using a global map with four possible levels of productivity (Löfgren 1984). In our study we calculated ocean productivity from a map that estimated chlorophyll concentration (grams of carbon m^{-2}) from visible light reflectance (SEAWIFS: Estimated Primary Productivity map, http://marine.rutgers.edu/opp/swf/Production/results/all2_swf.html) and with approximately 450 levels of productivity. On the one hand, using Fairbairn and Shine's species only, the correlation between our data on SSD and theirs is highly significant (mean SSD value from their populations, $r = -0.925$, $p = 0.001$, $n = 37$); this strengthens the view that the two SSD datasets are comparable. On the other hand, Fairbairn and Shine's ocean productivity data were not correlated with our data on ocean productivity ($r = -0.214$, $p = 0.217$, $n = 35$). Taken together, we conclude that the main difference between the results of Fairbairn and Shine and ours is due to the differences in ocean productivity data.

However, we argue that Fairbairn and Shine's own results do not support the differential niche-utilisation hypothesis. First, Fairbairn and Shine (1993) show that males tend to be larger than females in seabird populations (i) that have large average body mass and (ii) that breed in areas of high ocean productivity. This relationship, however, is the opposite of what may be predicted by the differential niche-utilisation hypothesis: more dimorphic taxa should be found in areas of low ocean productivity, whilst more monomorphic taxa should inhabit areas of high ocean productivity. Second, Fairbairn and Shine (1993) demonstrate that body size, SSD and Carbon fixation are tightly correlated and using multivariate analyses to separate highly correlated linear variables may not be relevant due to colinearity.

We conclude that sexual selection influences SSD in seabirds, although this influence appears to be weaker than that in several avian taxa such as shorebirds, bustards and North American blackbirds. Our results, however, do not support the fecundity selection and the differential niche-utilisation hypotheses. Further research should tease apart the correlates of male–male competition and female choice, use directional methods to separate cause and effect and provide better data for testing the differential niche-utilisation, for instance, by using direct estimates on the availability of prey species around feeding areas throughout the year and information on sex specific feeding strategies.

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Appendix 1. Data used in the analyses and data sources. GR, ground display; GA, ground and aerial display; MA, mainly aerial display. Ocean primary productivity values. References: (1) Awkerman 2004 pers. comm., (2) Causey 2002, (3) Cramp and Simmons 1977, (4) Cushman-Murphy and Pennoyer 1952, (5) Diamond and Schreiber 2002, (6) Dunning 1983, (7) Evans and Knopf 1993, (8) Fleet 1974, (9) Gauger-Metz and Schreiber 2002, (10) Gould et al. 1974, (11) Harris 1970, (12) Hatch and Nettleship 1998, (13) del Hoyo et al. 1992, (14) Huntington et al. 1996, (15) Imber 1976, (16) Jeremy and Weseloh 1999, (17) Johnsgard 1993, (18) Jouventin et al. 1999, (19) Jouventin and Bried 2001, (20) Marchant and Higgins 1990, (21) Megyesi and O'Daniel 1997, (22) Metz and Schreiber 2002, (23) Monroe and Sibley 1993, (24) Nelson 1978, (25) Nelson 2002, (26) Norman and Brown 1987, (27) Provincial Museum of Alberta, Blue-footed booby, http://www.pma.edmonton.ab.ca/vexhibit/ eggs/vexeggs/wrldeggs/bfboob.htm (28) Rand 1960, (29) Rutgers, The State Univ. of New Jersey Inst. of Marine and Coastal Sciences, http://marine.rutgers.edu/ opp, (30) Schreiber et al. 1996, (31) Schreiber and Burger 2002, (32) Serventy et al. 1971, (33) Simons 1985, (34) Simons and Hodges 1998, (35) Tickell 2000, (36) Urban et al. 1986, (37) Whittow 1993a, (38) Whittow 1993b, (39) Whittow 1997, (40) Williams 1995.

Species	Male mass in g	Female mass in g	Clutch size	Egg size index (length in mm × breadth in mm)	Display type	Ocean productivity (g C m^{-2})	References
Aptenodytes patagonicus	16000.0	14300.0	1.00	104.10 × 73.90	GR	152	13, 20, 23, 29, 31, 40
Bulweria bulwerii	107.1	99.9	1.00	41.21 × 30.31	GR	180	13, 20, 21, 23, 29, 31
Calonectris diomedea	955.6	817.3	1.00	69.00 × 45.00	GR	180	3, 13, 23, 29, 31
Daption capense	442.0	407.0	1.00	61.20 × 42.60	GA	094	13, 20, 23, 29
Diomedea amsterdamensis	6970.0	6120.0	1.00	121.00 × 76.00	GR	141	13, 23, 29, 31, 35
Diomedea bulleri	3120.0	2780.0	1.00	102.16 × 66.34	GR	178	13, 20, 23, 29, 31, 32, 35
Diomedea cauta	4350.0	3700.0	1.00	105.00 × 67.00	GR	152	13, 20, 23, 29, 31, 35
Diomedea chrysostoma	3900.0	3870.0	1.00	106.00 × 68.00	GA	094	13, 20, 23, 29, 31, 35
Diomedea epomophora	8840.0	7560.0	1.00	126.50 × 78.50	GA	128	13, 20, 23, 29, 31, 32, 35
Diomedea exulans	9110.0	7270.0	1.00	133.40 × 81.00	GA	243	13, 18, 20, 23, 29, 31, 32, 35
Diomedea immutabilis	3310.0	2990.0	1.00	107.60 × 68.60	GR	134	13, 23, 29, 35, 38
Diomedea irrorata	3750.0	3040.0	1.00	105.76 × 69.06	GR	369	1, 6, 13, 23, 29, 31
Diomedea melanophris	3710.0	3170.0	1.00	104.00 × 66.00	GA	094	13, 20, 23, 29, 31
Diomedea nigripes	3400.0	2990.0	1.00	108.00 × 70.00	GR	134	13, 20, 23, 29, 37
Eudyptes chrysocome	2500.0	2440.0	2.00	70.50 × 53.70	GR	128	13, 20, 23, 29, 40
Eudyptes chrysolophus	4760.0	5210.0	2.00	70.60 × 49.10	GR	082	13, 20, 23, 29, 40
Fregata andrewsi	1400.0	1550.0	1.00		GA	143	13, 20, 23, 29, 31
Fregata ariel	754.0	858.0	1.00	64.00 × 44.00	GA	093	6, 13, 20, 23, 29, 31
Fregata magnificens	1281.0	1667.0	1.00	68.00 × 47.00	GA	323	5, 6, 13, 23, 29, 31
Fregata minor	1239.0	1630.0	1.00	67.00 × 47.40	GA	376	9, 13, 20, 22, 23, 29, 31
Fregetta grallaria	47.0	52.0	1.00	36.30 × 26.40	GA	317	13, 20, 23, 29, 31
Fregetta tropica	51.7	54.2	1.00	37.00 × 27.00	GA	106	13, 20, 23, 29
Fulmarus glacialis	884.0	706.0	1.00	74.00 × 51.00	GR	450	3, 6, 12, 13, 23, 29
Fulmarus glacialoides	845.0	745.0	1.00	75.00 × 50.00	GR	056	13, 20, 23, 29, 31
Garrodia nereis	34.0	34.0	1.00	31.20 × 23.20	GR	302	13, 20, 23, 29, 31
Macronectes giganteus	5140.0	4220.0	1.00	104.90 × 65.70	GA	077	6, 13, 20, 23, 29
Macronectes halli	4902.0	3724.0	1.00	104.30 × 65.40	GA	152	13, 20, 23, 29, 31, 36
Oceanites oceanicus	33.6	36.2	1.00	34.90 × 24.50	MA	094	13, 20, 23, 29, 31
Oceanodroma leucorhoa	45.3	45.4	1.00	33.00 × 24.00	GA	266	13, 14, 20, 23, 29, 31
Pachyptila desolata	160.0	153.0	1.00	47.10 × 34.60	GR	094	13, 20, 23, 29
Pachyptila turtur	141.4	136.8	1.00	45.10 × 32.60	GR	178	13, 20, 23, 29
Pagodroma nivea	341.0	293.0	1.00	59.00 × 42.00	GA	094	13, 19, 20, 23, 29, 31
Pelagodroma marina	40.2	41.4	1.00	35.90 × 26.00	MA	411	3, 13, 23, 29
Pelecanoides urinatrix	110.3	101.0	1.00	40.20 × 31.60	GA	268	13, 20, 22, 23, 26, 29, 31
Pelecanus erythrorhynchos	6920.0	4970.0	2.00	87.10 × 57.10	GA	382	7, 13, 17, 23, 29, 31
Pelecanus occidentalis	3290.0	2824.0	2.60	73.00 × 45.40	GR	413	13, 17, 23, 29
Pelecanus onocrotalus	11450.0	7590.0	2.00	94.00 × 59.00	GR	145	3, 13, 17, 23, 29, 31
Pelecanus rufescens	5970.0	4920.0	1.99	82.10 × 54.60	GR	450	13, 17, 23, 29, 31, 36
Phaethon rubricauda	218.7	220.2	1.00	67.30 × 48.10	GA	108	8, 10, 13, 20, 23, 29
Phalacrocorax auritus	2453.0	2056.0	4.00	61.22 × 38.58	GA	448	13, 16, 17, 23, 29, 31
Phalacrocorax capensis	1171.0	1142.0	2.40	55.00 × 35.00	GR	450	13, 17, 23, 28, 29, 31
Phalacrocorax capillatus	3171.4	2525.0	3.00			405	13, 17, 23, 29
Phalacrocorax carbo	2400.0	2000.0	4.10	63.00 × 40.00	GR	243	13, 20, 23, 29, 31
Phalacrocorax chalconotus	2717.0	1813.6	2.50	66.00 × 42.00	GR	249	13, 20, 23, 29
Phalacrocorax magellanicus	1553.0	1417.0	3.00	62.00 × 38.00	GR	450	13, 17, 23, 29, 31
Phalacrocorax melanoleucos	800.0	700.0	4.00	47.00 × 32.00	GR	243	13, 20, 23, 29
Phalacrocorax pelagicus	2034.0	1702.0	3.00	58.00 × 37.30	GR	382	13, 17, 23, 29, 31
Phalacrocorax punctatus	1210.0	1160.0	2.70	59.40 × 36.80	GR	411	13, 20, 23, 29, 31
Phalacrocorax purpurascens	3320.0	2700.0	2.74	64.00 × 40.00	GR	077	13, 20, 29
Phalacrocorax sulcirostris	1100.0	900.0	5.00	47.00 × 33.00	GR	243	13, 17, 20, 23, 29, 31
Phalacrocorax urile	2428.1	1874.4	3.08	61.40 × 37.40	GR	382	2, 13, 17, 23, 29, 31
Phalacrocorax varius	1800.0	1400.0	3.32	59.00 × 38.00	GR	428	13, 17, 20, 23, 29
Phoebetria fusca	2800.0	2700.0	1.00	103.10 × 65.10	GA	152	13, 20, 23, 29, 31
Procellaria aequinoctialis	1390.0	1280.0	1.00	82.90 × 53.70	GA	094	13, 20, 23, 29
Procellaria parkinsoni	723.0	682.0	1.00	69.30 × 50.50	GR	360	13, 20, 23, 29, 31

Appendix 1 (*continued*)

Species	Male mass in g	Female mass in g	Clutch size	Egg size index (length in mm × breadthin mm)	Display type	Ocean productivity (g C m^{-2})	References
Procellaria westlandica	1232.5	1176.0	1.00	81.10 × 55.60	GR	450	13, 20, 23, 29
Pterodroma brevirostris	246.0	230.3	1.00	57.40 × 44.90		243	13, 20, 23, 29
Pterodroma inexpectata	302.0	335.1	1.00	60.53 × 43.90	MA	253	13, 20, 23, 29, 31
Pterodroma macroptera	668.0	667.0	1.00	67.50 × 48.30	MA	057	4, 6, 13, 15, 20, 23, 29, 31
Pterodroma nigripennis	169.6	165.9	1.00	51.00 × 37.00	MA	302	13, 20, 23, 29
Pterodroma phaeopygia	430.0	429.0	1.00	65.00 × 45.00	GA	376	11, 13, 23, 29, 31, 33, 34
Puffinus bulleri	345.0	306.5	1.00	65.44 × 42.96	GR	411	13, 20, 23, 29, 31
Puffinus pacificus	457.0	474.6	1.00	61.40 × 41.10	GR	317	13, 20, 23, 29, 31, 39
Pygoscelis antarctica	4435.0	3876.0	2.00	67.20 × 52.00	GR	396	13, 20, 23, 29, 40
Pygoscelis papua	5860.0	5070.0	2.00	68.10 × 57.70	GR	094	13, 20, 23, 29, 31
Papasula abbotti	1472.6	1491.5	1.00	82.00 × 53.00	GR	094	13, 20, 23, 29, 31
Morus bassanus	2932.0	3067.0	1.00	79.00 × 50.00	GA	450	13, 23, 24, 25, 29, 31
Morus capensis	2665.0	2608.0	1.00	76.13 × 48.22	GR	450	13, 20, 23, 24, 29, 31, 36
Sula dactylatra	2000.0	2533.3	2.00	66.30 × 46.50	GA	210	13, 20, 23, 29, 31
Sula leucogaster	1188.6	1343.6	2.00	61.00 × 40.00	GA	248	13, 20, 23, 29, 31
Sula nebouxii	1283.0	1801.0	2.00	57.00 × 41.00	MA	376	6, 13, 23, 24, 27, 29, 31
Sula sula	928.0	1068.0	1.00	60.80 × 41.00	GA	245	13, 20, 23, 29, 30, 31
Thalassoica antarctica	663.0	627.0	1.00	70.00 × 48.80	GA	056	6, 13, 20, 23, 29, 36

Chapter 7. *The influence of sexual selection and male agility on sexual size dimorphism in bustards (Otididae)*

Gina Raihani, Tamás Székely, M. Alejandro Serrano-Meneses, Christian Pitra & Paul Goriup (2006)

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Details and extent of the contributions by authors

G. Raihani: collected the data used in the study, manuscript writing (50%)

T. Székely: suggestions to improve the manuscript, general editing of the manuscript (15%)

M. A. Serrano-Meneses: phylogenetic analyses (15%)

C. Pitra: provided the phylogeny, suggestions to improve the manuscript (10%)

P. Goriup: scored mating system and display behaviour, provided some data on body size (10%)

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The influence of sexual selection and male agility on sexual size dimorphism in bustards (Otididae)

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Bustards vary considerably in sexual size dimorphism (SSD), ranging from reversed-dimorphic species (i.e. male < female) through monomorphic species to species in which an adult male can be three times heavier than an adult female. We used this unusual interspecific variation to test functional hypotheses of SSD using phylogenetic comparative methods. We found a strong allometric relation between SSD and body size that is consistent with Rensch's rule. We then tested whether the intensity of mating competition and the agility of male displays relate to SSD. First, the intensity of mating competition hypothesis predicts that males should be larger than females in species in which the males compete intensely for mates. As predicted, evolutionary changes towards more polygynous mating systems in bustards were associated with relatively larger males. Second, our results are also consistent with the aerial agility hypothesis, since in agile bustards the males tend to be smaller than females, whereas in nonagile bustards the males are usually larger. We also found that these two types of sexual selection have independent and statistically significant influences on SSD. We conclude that SSD in bustards is most consistent with sexual selection, and is influenced by both the intensity of sexual selection and the agility of male displays. Other hypotheses, however, such as fertility selection acting on females and differential use of niches by males and females remain untested.

Sexual size dimorphism (SSD) is one of the most conspicuous differences between the sexes. Several functional hypotheses have been proposed to explain SSD (reviewed in Hedrick & Temeles 1989; Shine 1989; Andersson 1994). First, directional selection may act on males (or on females). Sexual selection via male–male competition or female choice favours large male size in several birds and mammals (Webster 1992; Mitani et al. 1996; Dunn et al. 2001; McElligott et al. 2001; Lindenfors et al. 2003). Thus, an increase in body size may be particularly advantageous to males in polygynous species (Clutton-Brock & Harvey 1977; Owens & Hartley 1998), in which the intensity of sexual selection is greater because of increased competition between males over females.

Second, the direction of sexual selection on body size may also depend on whether the contest takes place on the ground or in the air (Payne 1984; Jehl & Murray 1986; Figuerola 1999). For example, small and agile males may have an advantage in contests that take place in the air (Andersson & Norberg 1981), and females also prefer small acrobatic males to large (or less acrobatic) males in raptors and shorebirds (Grønstøl 1996; Hakkarainen et al. 1996; Blomqvist et al. 1997; Figuerola 1999).

Third, disruptive selection may influence the body sizes of males and females towards different evolutionary optima. For instance, if males and females compete for resources, then each sex may benefit from avoiding extensive overlap with the other ('different niche utilization', Selander 1966; Shine 1989; Thom et al. 2004). However, it is difficult to distinguish whether differential niche

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utilization is a cause or a consequence of SSD. Thus, the exploitation of different resources may help maintain SSD, although it is unlikely to be the driving force behind it (Thom et al. 2004). Recent comparative studies have adopted a broad approach by investigating the influences of both sexual selection and ecological processes on SSD (Figuerola 1999; Székely et al. 2000; Perez-Barbería et al. 2002).

Many animal taxa show an allometric relation between body size and the extent of SSD across species (Rensch 1960). This relation, termed 'Rensch's rule' (Abouheif & Fairbairn 1997; Fairbairn 1997), states that among closely related taxa SSD increases with body size in those species in which the male is larger than the female, whereas SSD decreases with size in species in which the female is the larger sex. A recent genetic simulation model suggests that SSD may change rapidly in response to divergent selection pressures without significant long-term change in the genetic correlation between the sexes (Reeve & Fairbairn 2001).

Bustards (Otididae) are an excellent group with which to investigate SSD (Dale 1992), since they have one of the largest size dimorphisms in any avian taxa. In addition, they include species that have male-biased (e.g. great bustard, *Otis tarda*) and female-biased (e.g. lesser florican, *Sypheotides indicus*) dimorphism. Their mating behaviour ranges from socially monogamous to lek breeding. In the lek-breeding great bustard large males gain about 30% in body mass at the onset of the mating season, which suggests that large body mass enhances mating success (Carranza & Hidalgo-Trucios 1993), although it may also allow males to store reserves for the period of intensive displays. Male bustards also show an unusual range of display behaviour from spectacular ground displays by great bustards to highly acrobatic displays by red-crested bustards, *Lophotis ruficrista*. Finally, a recent molecular phylogeny provides a phylogenetic framework for comparative analyses (Pitra et al. 2002). Phylogenetic comparative methods are often used to test functional hypotheses of SSD (Owens & Hartley 1998; Székely et al. 2000; Dunn et al. 2001). These methods are useful for comparing traits across species or taxa within a statistical framework that controls for the effects of common ancestry.

We had two objectives in this study: first, to test whether SSD relates to body size in bustards as expected from Rensch's rule and second, to test two mutually nonexclusive functional hypotheses of SSD. The intensity of mating competition hypothesis predicts that males should be larger than females in species in which males compete intensely for mates, whereas the display agility hypothesis predicts that males should be relatively smaller than females in species with aerial displays compared with species with nonagile male displays, since reduced body size is assumed to enhance agility.

METHODS

Data and Phylogeny

We compiled data on body size, mating behaviour and male agility from primary research publications, handbooks and unpublished information (Appendix 1). Social mating system, a proxy for the intensity of mating competition, was scored as monogamy, territorial polygyny and lek polygyny. We assumed that mating competition increases from monogamy (score 1) to lek polygyny (score 3, see similar scoring by Székely et al. 2000; Dunn et al. 2001). Three species showed both territorial polygyny and lekking, so were scored 2.5 (Appendix 1). Our scores were highly correlated with an independent scoring of mating system (Dale 1992; $r_S = 0.901$, $N = 17$ species, $P < 0.0001$). Male agility was scored between 1 (low) and 5 (high, Appendix 1). This scoring of male agility is consistent with the approach of Figuerola (1999) and Székely et al. (2000). The score of 1.5 was given when it was uncertain whether male display was 1 or 2 (Appendix 1). The distinctions between scores 1, 1.5 and 2 are ambiguous, so we took the advice of an anonymous referee and combined these scores in the analyses. Note that using the original scores (Appendix 1) does not change our major conclusions.

Initially, we attempted to collect data on body mass of bustards as a further proxy for body size. Mass data, however, are subject to daily and seasonal fluctuations (Carranza & Hidalgo-Trucios 1993; Witter & Cuthill 1993). In addition, sex-specific mass data were available for only a few species. We therefore omitted body mass from the analysis.

The bustard phylogeny was provided by a recent molecular study (Pitra et al. 2002) that used sequences from the mitochondrial cytochrome *b* gene, the noncoding mitochondrial control region II and an intron–exon crossing fragment of the nuclear chromo-helicase-DNA binding gene. We augmented this phylogeny with two species (Karoo bustard, *Eupodotis vigorsii*, and the little brown bustard, *Eupodotis humilis*). The phylogenetic position of the latter two species was provisionally allocated as sister taxa to Rüppell's bustard, *Eupodotis rueppellii*, because both the Karoo bustard and the little brown bustard were formerly placed in a separate genus (*Heterotetrax*) together with Rüppell's bustard (Snow 1978).

Phylogenetic Comparative Analyses

We used the phylogenetic independent contrasts method of Felsenstein (1985) as implemented by CAIC (Purvis & Rambaut 1995) to control for phylogenetic nonindependence of species. Wing length (mm) was log transformed prior to the analyses. An assumption of Felsenstein's method is that standardized contrasts should be independent from their estimated nodal values. We verified this assumption by plotting standardized contrasts against the estimated nodal values for our variables: none of these correlations were statistically significant.

We tested the allometric relation between male and female body size by fitting major axis regressions (Sokal & Rohlf 1981) using either the species data or the phylogenetic independent contrasts (Garland et al. 1992). Rensch's rule predicts that the slope of male:female size should be significantly larger than 1. The major axis regression of phylogenetic independent contrasts was forced

through the origin (Harvey & Pagel 1991). We provide the slope of major axis regressions (b) and the 99% confidence intervals (lower CI–upper CI) that were calculated by bootstrapping the contrasts.

We calculated SSD as contrasts in log (male wing) − contrasts in log (female wing). It is customary to use log-transformed data for calculating SSD since the differences between males and females expressed as logarithms provide more accurate estimates of SSD than ratios do. Log transformation of sizes also makes the more reasonable assumption that different lineages are equally likely to make the same proportional change in size (Purvis & Rambaut 1995). Least-squares regressions of contrasts were forced through the origin, because the model predicts the mean value of independent contrasts to be zero (Harvey & Pagel 1991). We investigated the relations between SSD (dependent variable), the intensity of mating competition and male agility (independent variables) in bivariate and multivariate least-squares regressions. Evolutionary changes in agility and the intensity of mating competition were not related (Pearson correlation: $r_{24} = 0.136$, $P = 0.516$). We also tested the interaction term between the intensity of sexual selection and male agility (with SSD as the dependent variable). This interaction was not significant ($N = 24$ contrasts, $P = 0.484$), so we excluded the interaction term from the final multivariate regression model. In the multivariate model we provide the partial correlation coefficient r_p, the significance of r_p and effect sizes (partial η^2). We used SPSS version 11.00 (SPSS Inc., Chicago, IL, U.S.A.) for statistical calculations, except that bootstrapping of confidence intervals for major axis regression was carried out in R (Ihaka & Gentleman 1996).

RESULTS

Rensch's Rule

Our results are consistent with Rensch's rule since male-biased SSD was greater in large bustards than in small ones, and the 99% confidence intervals did not include 1 (Fig. 1a). These results remained statistically significant when we used phylogenetically independent contrasts (Fig. 1b).

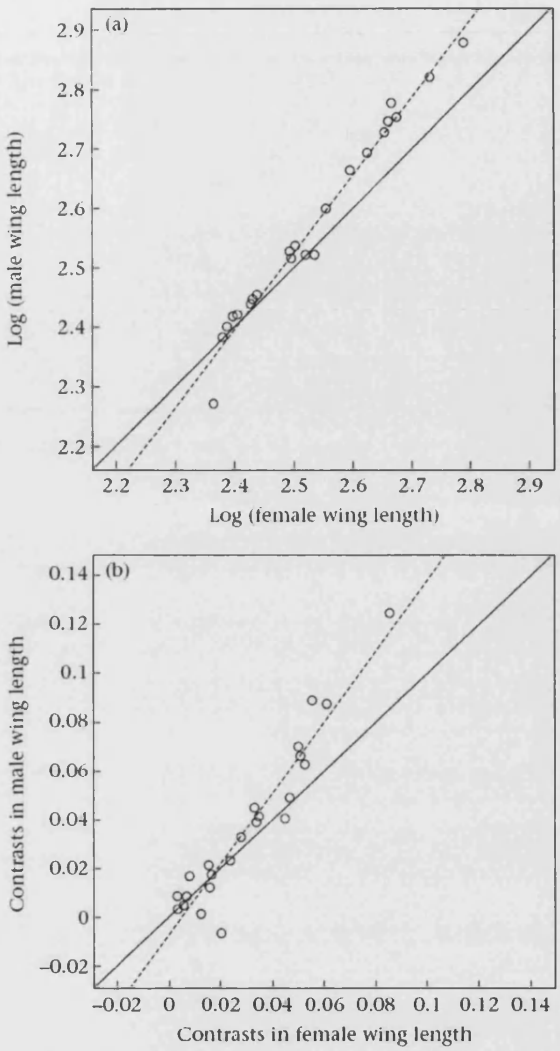

Figure 1. Rensch's rule in bustards. The continuous line indicates the isometric relation and the dotted line represents the fitted relation between male size and female size by major axis regression for (a) species ($b = 1.311$, 99% confidence intervals 1.204–1.430, $N = 25$ species) and (b) phylogenetic contrasts ($b = 1.542$, 99% confidence intervals 1.218–1.846, $N = 24$ contrasts).

Intensity of Mating Competition and Male Agility

Evolutionary changes in SSD were positively correlated with changes in the intensity of mating competition (Fig. 2a). Since the contrasts were highly variable around zero, we repeated the analysis by excluding zero contrasts (see a similar approach by Owens & Hartley 1998). Nevertheless, the relation remained significant ($r = 0.645$, $F_{1,9} = 6.395$, $P = 0.032$).

Evolutionary changes towards agile displays were also correlated with changes towards smaller males relative to females (Fig. 2b). The strength of the relation remained after we excluded zero contrasts, although it was not statistically significant ($r = -0.424$, $F_{1,11} = 2.413$, $P = 0.149$).

In the multivariate model ($r^2 = 0.431$, $F_{2,22} = 8.342$, $P = 0.002$), both the intensity of mating competition and male agility were associated with SSD. Evolutionary changes towards larger males relative to females were associated with both intensified mating competition ($r_p = 0.563$, $F_{1,22} = 10.197$, $P = 0.004$) and reduced agility of male displays ($r_p = -0.533$, $F_{1,22} = 8.749$, $P = 0.007$). Effect sizes of mating competition and display behaviour were partial $\eta^2 = 0.317$ and 0.285, respectively.

DISCUSSION

Our study confirms Rensch's rule in bustards (Payne 1984), and adds to a growing number of taxa in which the rule has been shown, including mites, lizards,

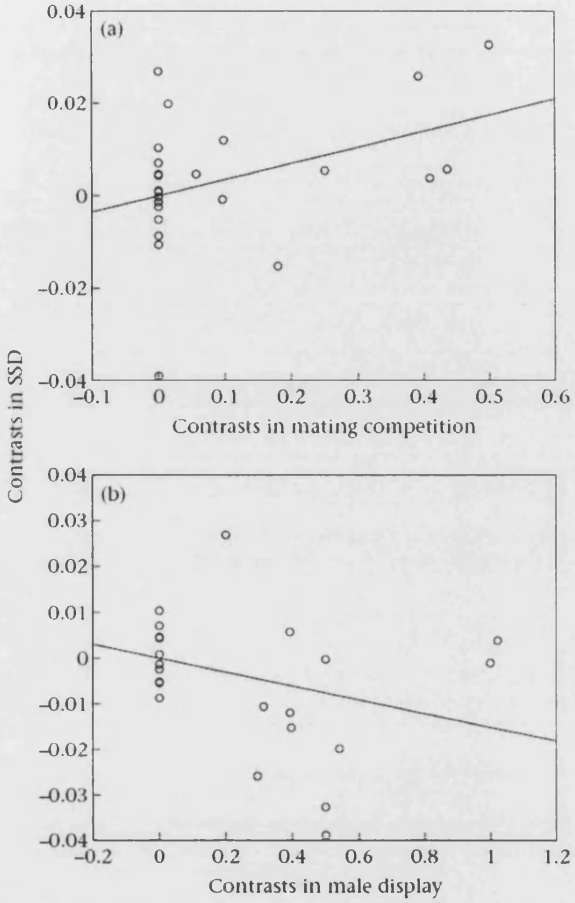

Figure 2. Phylogenetically independent contrasts in sexual size dimorphism (SSD) and (a) the intensity of mating competition ($r = 0.453$, $F_{1,23} = 5.934$, $P = 0.023$) and (b) male agility ($r = -0.409$, $F_{1,23} = 4.634$, $P = 0.042$). Regressions were forced through the origin.

hummingbirds and shorebirds (Abouheif & Fairbairn 1997; Cullum 1998; Colwell 2000; Székely et al. 2004). Székely et al. (2004) have shown that the rule is driven by the interaction between mating competition and male display behaviour in shorebirds. In bustards, however, this interaction was not significant ($P = 0.484$). We suggest that the latter result may be caused by the small number of bustard species in the analysis, which limits the statistical power of models. Note, however, that the predictive power of multivariate models was comparable between bustards ($r^2 = 0.431$) and shorebirds ($r^2 = 0.476$–0.454, Székely et al. 2004).

The relation between mating competition and SSD is consistent with the hypothesis that sexual selection is responsible for the large body size in males of polygynous species. This relation has been reported in New World blackbirds (Icteridae), shorebirds (Charadrii) and a wide range of other avian taxa (Webster 1992; Székely et al. 2000; Dunn et al. 2001). Thus, male–male competition and female choice may lead to an evolutionary increase in overall body size. Fertility selection is unlikely to explain SSD given that most bustard species lay only small clutches of one or two eggs (Collar 1996).

Our results are also consistent with the aerial agility hypothesis. The influence of agility was comparable to that of mating competition, as indicated by the partial effect sizes. Sexual selection will favour small males in those species that display aerially (Andersson & Norberg 1981; Jehl & Murray 1986). This relation has also been reported in nonpolyandrous shorebirds with reversed SSD, showing a significant reduction in male body size from species with less acrobatic to those with more acrobatic displays (Figuerola 1999; Székely et al. 2000). Evidence that females prefer small males also exists for moorhens, *Gallinula chloropus*, and fruit flies, *Drosophila subobscura* (Petrie 1983; Steele & Partridge 1988).

Future work may expand our study by investigating other morphological traits that relate to body size, for instance body mass and tarsus length. In addition, body size appears to vary between bustard populations, thus comparing populations may be a fruitful approach. Work is also needed to quantify the ecology, mating behaviours and display behaviours of bustards, most species of which are threatened.

Various selection pressures may act on body sizes of females and males. For instance, in most bustards the incubation of eggs and rearing of young are left entirely to the female (Collar 1996). This may select for smaller body size in females, since they would be better covered by vegetation during incubation and less conspicuous to predators. Large size, however, may allow the sexes to accumulate fat and buffer them against fluctuations in food resources. Thus, if one sex forages in a more variable environment than the other, the sexes may have different optimal body sizes.

The colours and pattern of plumage may amplify display behaviour, and thus play an important part in the evolution of SSD. Display behaviour appears to correlate with male coloration in bustards, since species with aerial displays have more melanin in their plumage (Dale 2006). The latter result is consistent with the findings of Bókony et al. (2003), who showed that more melanistic shorebirds have more agile displays. Thus, a further implication of aerial displays in males is that their plumage is more conspicuous against the light background of the sky.

In conclusion, our study shows that SSD in bustards is consistent with Rensch's rule. Our results also support the sexual selection hypothesis that directional selection may either increase or decrease body size of males, depending on the particular mating strategy and display type.

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Appendix 1

Table A1. Data sources

Species	Male wing (mm)	Female wing (mm)	Mating system	Display type	Source
Afrotis afra	281.0 (47)	270.0 (23)	PG	4	4
Afrotis afraoides	285.6 (18)	274.5 (13)	PG	5	1
Ardeotis arabs	604.0 (7)	496.7 (6)	PG	2	1
Ardeotis australis	569.0 (6)	474.0 (12)	PG	2	3
Ardeotis kori	758.0 (36)	616.0 (46)	PG	2	5
Ardeotis nigriceps	661.2 (12)	539.5 (11)	PG	2	1
Chlamydotis undulata	399.2 (25)	359.2 (26)	PG	3	1
Eupodotis caerulescens	333.5 (8)	330.8 (4)	MG	1	1
Eupodotis humilis	252.6 (10)	245.1 (8)	MG	1	1
Eupodotis rueppellii	329.0 (18)	313.0 (13)	MG	1	4
Eupodotis senegalensis	276.1 (14)	268.5 (4)	MG	1	1
Eupodotis vigorsii	337.4 (5)	312.3 (7)	MG	1	1
Houbaropsis bengalensis	333.0 (15)	343.4 (14)	PG	4	1
Lissotis hartlaubii	337.8 (10)	310.8 (6)	PG/LEK	1	1
Lissotis melanogaster	345.5 (26)	319.3 (23)	PG/LEK	4	1
Lophotis gindiana	262.8 (18)	250.1 (12)	PG	5	1
Lophotis ruficrista	263.9 (12)	254.5 (11)	PG	5	1
Lophotis savilei	243.0 (9)	240.8 (4)	PG	5	1
Neotis denhami	558.0 (11)	459.0 (7)	PG/LEK	2	5
Neotis heuglinii	495.0 (3)	423.0 (4)	PG	1.5	2
Neotis ludwigii	536.0 (6)	452.0 (5)	PG	1	4
Neotis nuba	463.5 (2)	395.0 (4)	PG	1.5	4
Otis tarda	600.1 (12)	464.8 (10)	LEK	2	1
Sypheotides indicus	187.8 (12)	231.7 (11)	PG	5	1
Tetrax tetrax	252.0 (29)	249.0 (15)	PG	4	2

The numbers of individuals (males and females) measured for each species are given in parentheses. Mean wing lengths were calculated from the unpublished measurements of P. Goriup and P. Osborne, where indicated. Social mating system and display behaviour were scored by P. G. from unpublished notes and observations. Mating system was scored as monogamy (MG), territorial polygyny (PG) and lek polygyny (LEK). Display type was scored as (1) ground display with vocalization only and/or erect neck display; (2) ground display; males inflate their oesophagus and reveal the underparts of the plumage; (3) ground display with running and occasional leaps in the air; (4) aerial nonacrobatic display of high flying; (5) aerial acrobatic rocket display (Johnsgard 1991). Note that display scores 1, 1.5 and 2 were combined in statistical analyses (see Methods). Source: (1) P. D. Goriup & P. E. Osborne (data compilers); (2) Johnsgard 1991; (3) Marchant & Higgins 1993; (4) Urban et al. 1986; (5) Percy FitzPatrick Institute of African Ornithology: http://web.uct.ac.za/depts/fitzpatrick/docs/fam31.html.

Appendix. *Sexual selection as the possible underlying force in Calopterygid wing pigmentation: comparative evidence with Hetaerina and Calopteryx genera*

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Odonatologica in press

Details and extent of the contributions by authors

M. A. Serrano-Meneses: quantification of wing pigmentation, manuscript writing (50%)

G. Sánchez-Rojas: statistical analyses (20%)

A. Córdoba-Aguilar: supervision of the study, insect collection, suggestions to improve the manuscript (30%)

Abstract

One of the most striking and conspicuous traits in some species of odonates is wing pigmentation. In the Calopterygidae, males bear species-specific wing pigmentation patterns. Recent evidence in different genera has suggested that sexual selection is the underlying hypothesis for this trait. However there are other alternative hypotheses for conspicuous traits which actually apply to wing pigmentation patterns. In this paper, we have advanced and tested two predictions to see whether the sexual selection hypothesis stands. We first compared the coefficients of variation of pigmentation against that of a selected set of other animals' traits that are maintained by natural and sexual selection. By using two species of *Hetaerina* (*H. americana* and *H. vulnerata*) and three of *Calopteryx* (*C. aequabilis, C. haemorrhoidalis*, *C. xanthostoma*) the aim was to see whether pigmentation variation is different from other traits particularly those maintained by sexual selection. The second prediction is that pigmentation should not differ in species whose populations are in sympatry (compared to allopatry) if sexual selection is driving pigmentation evolution (compared, for example, to an ecological character displacement hypothesis in which pigmentation between species should differ). For this, we compared the pigmentation of sympatric and allopatric populations of two species of *Hetaerina*, *H. americana* and *H. vulnerata*. For the first prediction the coefficient of variation of pigmentation did not differ from that of sexually selected traits in other animals, but was different from that of naturally selected traits. Also, the pigmentation of the two species in sympatry was not significantly different from each other which was also the case for allopatric populations. These results are in agreement with the sexual selection hypothesis as the mechanism of maintenance for pigmentation in these animals. Some other alternative hypotheses for the evolution of pigmentation (differences in habitat use in both sexes, warning to predators by males and ecological character displacement) are discussed in the light of these results.

1. INTRODUCTION

Darwin (1871) put forward the theory of sexual selection to explain the differences in morphology, physiology and behaviour between the sexes. These differences are frequently observed as traits that only males bear and whose expression is exaggerated. According to sexual selection theory, two processes have propelled the evolution of male exaggerated traits: males will compete with each other to have sexual access to females while females will choose among males to fertilise their eggs. More recently, however, sexual conflict - the differences between the sexes in reproductive interests - has been also proposed as another driving process in sexual selection (reviewed by Chapman et al. 2003).

Although sexual selection is the most powerful explanation for the evolution of exaggerated traits, some alternative hypotheses have also been advanced. One alternative is that both sexes differ in habitat use (the different habitat use hypothesis) which may have produced particular adaptations to certain environments (Andersson 1994). This may be the case, for example, if both sexes differ in the places where they forage and have evolved different traits to cope with distinct needs. A second explanation suggests that males may communicate to their predators that they are difficult targets via conspicuous traits that predators are able to recognise (the predator warning hypothesis; Baker & Parker 1979). A third hypothesis indicates that conspicuous traits (i.e. colour) may act as badges aimed to communicate social status to conspecifics (the social badge hypothesis; Andersson 1994). These badges would prevent unnecessary, potentially costly escalated contests for the access to resources that are not related to sexual reproduction directly or to females (reviewed by Andersson 1994). The last hypothesis, originally laid out by Brown & Wilson (1956), is that exaggerated traits are species-specific traits evolved to distinguish members of other species to prevent interspecific matings (the ecological character displacement hypothesis) in zones of distribution overlapping of two or more species.

Sexual selection has been studied in great detail in Odonata, particularly the Calopterygidae (reviewed by Córdoba-Aguilar & Cordero-Rivera 2005). Males of most species in this family develop specific wing pigmentation patterns soon after emergence (Silsby 2000). These patterns differ from one species to another (for example, a red basal colouration in the genus *Hetaerina* and metallic black in the genus *Calopteryx*) and are extravagant in the sense that they make males appear conspicuous. Once wing pigmentation has been developed, males start looking for places (= territories) to defend where females arrive at for copulation and oviposition (Corbet 1999). It is while staying at territories when wing pigmentation is apparently shown to conspecifics: to males during the flying contests for the acquisition or defence of a territory (suggested by Koskimäki et al. 2004; Rüppell et al. 2005; Contreras-Garduño et al. 2006), and to females during the pre-copulatory flying courtship (which is the case of *Calopteryx* only; reviewed by Córdoba-Aguilar & Cordero-Rivera 2005).

Several sources of evidence in the genera *Calopteryx* (i.e. Siva-Jothy 1999, 2000; Córdoba-Aguilar 2002; Córdoba-Aguilar et al. 2003; Rantala et al. 2000; Rolff & Siva-Jothy 2004; Svensson et al. 2004), *Hetaerina* (Grether 1996a, b; Contreras-Garduño et al. 2006) and *Mnais* (Plaistow & Tsubaki 2000; Tsubaki & Hooper 2004) strongly suggest that male wing pigmentation is sexually selected. Pigmentation has been associated with male-male competition for several reasons, the most important being that high pigmented males are usually the ones that defend a territory (i.e. Grether 1996a, b; Siva-Jothy, 2000; Córdoba-Aguilar 2002) and they do it for longer periods (i.e. Grether 1996a, b; Córdoba-Aguilar 2002) compared to less pigmented males. The underlying reason for this is that high pigmented males usually have more muscular thoracic fat (Contreras-Garduño et al. 2006) and fewer intestinal parasites (Siva-Jothy 2000; Córdoba-Aguilar 2002). A number of studies have shown that both variables are important in territorial competition. In terms of fat reserves, they provide the necessary energetic input during territorial aerial contests (Marden & Waage 1990; Plaistow & Siva-Jothy 1996); in the case of parasites, they have a negative impact on fitness by affecting the elaboration of fat reserves, therefore reducing male longevity (Siva-Jothy & Plaistow 1999). Male pigmentation has been also associated with female choice in

different *Calopteryx* species where females mate with males that have more pigmentation (Siva-Jothy 2000; Córdoba-Aguilar 2002). Highly pigmented males actually end up having a higher lifetime mating success compared to less pigmented males (Córdoba-Aguilar 2002).

Despite the above sexual selection evidence for the evolution of wing pigmentation in calopterygids, a potential drawback is that other alternative hypotheses can also explain the same phenomenon. For the case of the habitat difference use hypothesis, it is reasonable to accept that adult females and males differ in habitat use. Females, for example, perch on trees or forage away from the water while males stay near the water mainly to defend or try to obtain a territory (Corbet 1999). Feeding, for example, can be done in distinct places according to these differences. The predator warning hypothesis may also apply given that males exhibit themselves during territory defence and also because the territories are usually open spaces where active predators, such as birds, can enter (e.g. Krebs & Avery 1984). The social badge hypothesis may apply only if males establish complex social groups whose members respect owners of territories (Trivers 1985). This seems difficult to operate in calopterygids as territory ownership follows desperado rules: non-territorial males get an extremely small number of copulations so that males will fight until exhaustion if territories are not vacant (Plaistrow & Siva-Jothy 1996). Under this system, only those males that have more fat reserves, which are correlated with and, possibly, communicated via pigmentation (Contreras-Garduño et al. 2006), are the ones that will defend a territory (Marden & Waage 1990; Plaistow & Siva-Jothy, 1996). On the other hand, the last hypothesis – ecological character displacement - may also apply to explain the evolution of wing pigmentation, as interspecific matings and hybrids of different calopterygid species have been observed in nature (Dumont et al. 1987; Lindeboom 1993).

In this paper we examine the potential for the sexual selection hypothesis to explain the evolution of pigmentation in the light of two predictions. The first prediction is related to the expected phenotypic variation for sexually selected traits. According to sexual selection theory and although the underlying reason for this is unclear, traits that have

evolved via this pressure should exhibit considerably large variation in expression (Andersson 1994). This prediction assumes that the production of pigmentation is costly so that the different habitat use and the ecological character displacement hypotheses are discarded. However this is not the case for the predator warning hypothesis as only males in good condition would be able to afford the production of costly traits to communicate their predators about their ability to escape (following the handicap principle; Zahavi & Zahavi 1997). The second prediction is related to the expected pattern of pigmentation differences in allopatric versus sympatric populations. According to the ecological character displacement hypothesis, one would expect that populations (= pigmentation) of different species should be more different when in sympatry as the risk of interspecific mating is higher in these places compared to allopatry (see an example in Waage 1975, 1979). This difference, however, should not be the case if sexual selection is operating as males and females are able to recognise non-cospecifics.

We have tested these two predictions using members of two of the best studied genera in calopterygids, *Hetaerina* and *Calopteryx*. To test the first prediction, we have used the coefficient of variation in pigmentation (CV) expression in two species of *Hetaerina* and three species of *Calopteryx*, and compared it to that of traits from which there is available information as for whether they are evolutionarily shaped by sexual or natural selection in other taxa. This comparison allows seeing the boundaries (either in the natural or sexual selection "zone") in which the CV of pigmentation lies. As for the second prediction, we have used data of *H. americana* and *H. vulnerata* populations which have varying degrees of sympatry and allopatry in the central regions of Mexico. We compared how much different pigmentation is in sympatry and allopatry. Most *Hetaerina* species are convenient for this comparison as males show strikingly similar wing basal pigmentation patterns.

2. MATERIALS AND METHODS

(a) ***Pigmentation measurements and phenotypic expression patterns***

The following species and locations were used: *C. aequabilis* from Canada in 1999 (N = 18), *C. haemorrhoidalis* from Spain in 1998 (N = 135), *C. xanthostoma* from Spain in 1998 (N = 28), *H. americana* from Mexico in 2000 (N = 30) and *H. vulnerata* from Mexico in 2000 (N = 24). The following conditions had to be met for the collection: 1) animals had to be sexually mature so that pigmentation was already fully developed, and 2) males had to come from the same population, preferentially being collected on the same day to avoid morphological variation caused by seasonal differences (Corbet 1999). After capture animals were stored in 70 % ethanol. Each individual was then placed on a petri dish containing water for two hours for tissue rehydration. Water was removed by placing every individual on a dry cloth at room temperature. Wings were cut off from their insertion to the thorax, fixed on plastic acetates and secured with transparent tape. Drawings of both total wing and pigmented areas were produced for every individual by using a stereo microscope equipped with a drawing tube (*Zeiss* microscope, model *Stemi SV 6*). Drawings were made manually at a constant distance on waxed paper. The waxed condition reduces variation in weight due to humidity. Cutting outs of the pigmented and non-pigmented areas were done and weighed using an electronic analytical balance (*OHAUS*, model *CT200*; precision ± 0.001 g). These data were later used to estimate the pigmentation percentage for every individual. A similar method for measuring pigmentation has been used in previous studies and has produced reliable results (see Córdoba-Aguilar 2002). Of course, this methodology only uses size of pigmentation but other aspects such as intensity are omitted. However, given that size has been already correlated to sexual selection (i.e. Siva-Jothy 2000) and for intensity we have no information, this approach is still valid.

We obtained the CV for pigmentation areas. CV allows comparing data as it is expressed as a percentage (the lower the value of CV, the less variation is exhibited in the data; Zar 1999). We compared the CV of pigmentation with that of traits that have evolved by sexual and natural selection from other taxa gathered from different literature sources

(see Appendix 1 and Appendix 2 for a full list of these taxa and the traits used respectively). We first checked for the distribution shape of the CVs of naturally selected traits which did not depart from normality (Kolmogorov-Smirnov test, p = 0.58). Using the mean and standard deviation of these traits ($\mu = 4.51$, $\sigma = 1.87$), we then transformed the CV of pigmentation and that of the other traits that have evolved by sexual selection of other taxa, to a standard normal distribution (Zar 1999). With this calculation we obtained Z scores (or units of standard deviations) for the pigmentation CV, which allowed us to test whether these transformed values, including those of other sexually selected traits in other taxa (Figure 1a), were likely to be found under the normal curve of naturally selected traits (Figure 1a). This was done by checking the critical values of the proportion of the normal curve distribution in statistical tables (Zar 1999), which allowed us to obtain the proportion of the normal curve that lies beyond a given Z score. For example, if the p-value of a Z score of pigmentation equals or approaches zero, it means that the Z score lies outside the normal curve exhibiting a variation similar to that of sexually selected traits.

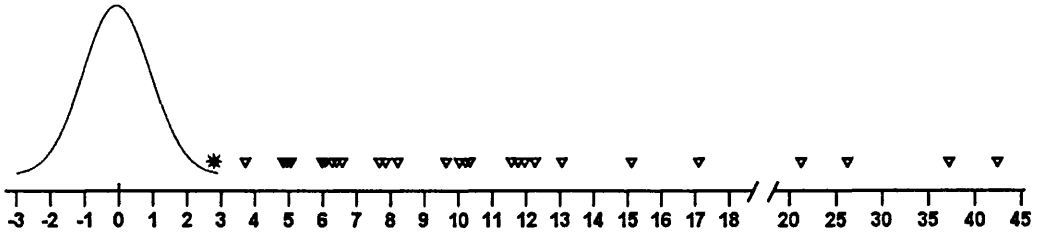

Figure 1a. Distribution of traits under natural and sexual selection in non-odonate species (see Appendix 1 and 2 respectively). The normal curve represents the distribution of the CVs of naturally selected traits and zero denotes the mean. Numbers are standard deviations. Note that up to 98% of the values of the CVs of traits under natural selection are included within ± 3 standard deviations. Due to higher variation, most of the traits under sexual selection (inverted triangles) fall outside the normal distribution curve and more than 3 standard deviations away from the mean (except for the extreme value noted by an asterisk).

(b) Pigmentation variation in allopatric and sympatric populations

The following specimens were collected in 2000 in Mexico: *H. americana* (N = 20) from Jiutepec, Morelos (allopatric population), *H. vulnerata* (N = 20) from Xalapa, Veracruz (allopatric population), *H. americana* (N = 20) and *H. vulnerata* (N = 20) from Jiutepec, Morelos (sympatric population). The collecting conditions were similar as indicated before. Pigmentation measurement, given that males of different species share similar patterns (a red basal spot on the basis of each wing), was measured as the longitudinal length of the spot (from the wing basis to the tip). The mean pigmentation sizes of allopatric and sympatric populations were tested using t-tests.

Data are provided as means ± STD unless stated otherwise.

3. RESULTS

(a) *Phenotypic expression of pigmentation*

The CVs of pigmentation (*C. aequabilis* = 13.02%, *C. haemorrhoidalis* = 14.89%, *C. xanthostoma* = 19.70%, *H. americana* = 14.44%, *H. vulnerata* = 13.06%) fell outside the curve of traits shaped by natural selection (Figure 1b) which is similar to what occurs to sexually selected traits in other taxa (compare Figure 1a and 1b).

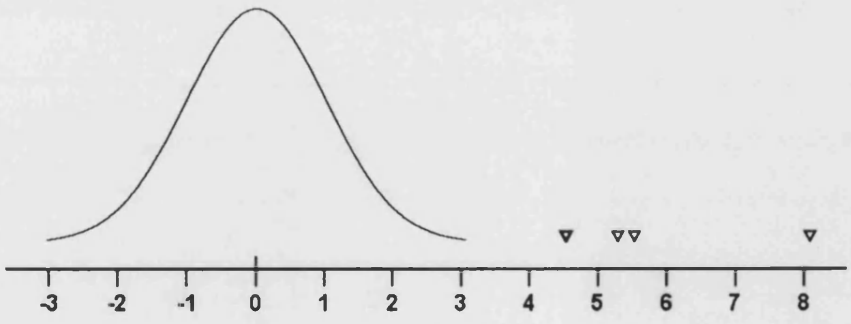

Figure 1b. Normal distribution curve of the CVs of traits under the influence of natural selection. Zero denotes the mean and numbers are standard deviations. Inverted triangles represent the Z scores of the CVs of pigmentation. Note that 2 symbols overlap at approximately 4.5 standard deviations.

This exclusion was statistically significant in all species (Table 1).

Table 1. Coefficients of variation of wing pigmentation, Z scores and the proportion of the area that lies beyond these scores. P values reflect how likely it is that the the expression of pigmentation values fell outside the distribution of natural selection characters.

Species	CVs of WP	Z Score	*p*
Calopteryx aequabilis	13.02	4.53	$p = 0.000$
Calopteryx haemorrhoidalis	14.89	5.53	$p = 0.000$
Calopteryx xanthostoma	19.70	8.09	$p = 0.000$
Hetaerina americana	14.44	5.29	$p = 0.000$
Hetaerina vulnerata	13.06	4.55	$p = 0.000$

(b) ***Pigmentation in allopatric and sympatric populations***

There were no significant differences between the lengths of the pigmented patches in allopatric ($t_{38} = -1.58, p = 0.120$) or sympatric populations ($t_{38} = 0.95, p = 0.350$).

4. DISCUSSION

Our results indicate that in a selected subset of species of *Calopteryx* and *Hetaerina*, as representatives of Calopterygidae, evidence supports a sexual selection explanation for the maintenance of wing pigmentation, a common trait shared by most members of this family. Members of these two genera have been traditionally used for testing sexual selection assumptions and actually it is mainly from *Calopteryx* where evidence for this theory is stronger (reviewed in Córdoba-Aguilar & Cordero-Rivera 2005). In *Calopteryx* and *Hetaerina*, variation in the phenotypic expression of pigmentation was more similar to that of traits that have been shaped by sexual selection than for natural selection in other taxa. The list of traits we used came from very different animals. Given this and the large sample of species used, is unlikely that our results can be due to chance. On the other hand, we also tested whether pigmentation was more different in sympatric

populations than in allopatric populations assuming that in the former, pigmentation should differ if an ecological character displacement process was selecting for more different pigmentation patterns. Contrary to this, we found that pigmentation size in two *Hetaerina* species did not differ between sympatric and allopatric populations. This clearly means that if ecological character displacement is operating, the selected trait that males and/or females will recognise is not pigmentation.

For the first prediction, the different habitat use hypothesis is unlikely to explain our results mainly because pigmentation is a trait that is costly to produce and there would be no point in evolving a character that, over time will become costly. The other reason is that although indeed mature males and females occupy different places, teneral and fully mature non-territorial males may actually forage also away from territories (Kirkton & Schultz 2001) where females probably are. The predator warning hypothesis may apply as long as pigmentation has evolved to become an honest character that not all individuals are able to produce so that the information provided to predators is a guarantee that the animal is indeed able to escape if chased. The likely predators that may apply to this extension are birds and other insects as they are actively looking for prey when foraging (reviewed by Corbet 1999). Given that pigmentation correlates with fat reserves (so that males with more pigmentation can devote more energy to fight; Contreras-Garduño et al. 2006), it is possible that more highly pigmented males can be better at evading a predator. No studies have been done in this respect. This hypothesis, however, assumes that predators forage only where males are present. Although there is evidence that some bird species specialize in eating odonates (i.e. Kennedy 1950; Bagg 1958), to our knowledege nobody has documented a male bias in dragonfly predation with these predators.

The results of our second prediction are incompatible with the ecological character displacement hypothesis. Other studies in *H. americana* have suggested that males use pigmentation expression when fighting for territories. These studies have found that males with more pigmentation are more likely to win figths (Köskimaki et al. 2004; Contreras-Garduño et al. 2006). This possibly means that contestants recognise each

other's fighting potential based on the size of the pigmented patches. On the other hand and contrary to this recognition idea, recent work in *C. virgo* and *C. splendens* has shown that, when in sympatry, the former species can be more aggressive towards the latter displacing it (Tynkkynen et al. 2004). Aggression is more directed against *C. splendens* males with larger wing pigmentation spots as the they look more similar to *C. virgo* pigmentation patterns. A similar lack of recognition of heterospecifics has been detected in *C. maculata* and *C. aequabilis* damselflies (Waage 1975, 1979): female pigmentation was different in sympatric places compared to allopatric places. Paradoxically, it is females and not males who have evolved different pigmentation patterns to avoid interspecific matings so males would discriminate among homo- and heterospecific female. Interestingly and not in support of the lack of recognition possibility, evidence in *C. xanthostoma* males suggests that they are able to recognize female mates from non mates (Hooper 1995). Including some other odonates in general, it is not clear whether and to what extent, males can recognize homo- and heterospecifics as it seems that not all species have this ability even in species of the same genus (reviewed by Corbet 1999). Given the potential costs of unnecessary fights by males and matings by females, this field deserves further research. What it seems a reasonable hypothesis for the origin of pigmentation with respect to the ecological character displacement hypothesis, is that pigmentation, given its production costs, arose via sexual selection and possibly is now being shaped by other evolutionary forces such as the interspecific male-male aggression observed in *Calopteryx* (Tynkkynen et al. 2004, 2005).

Finally our pigmentation measurements have been based on size only. However, given the fact that odonates can see ultraviolet patterns (Corbet 1999), the potential exists for using this means for communication. The potential role for this deserves further investigation.

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APPENDIX I

Coefficients of variation of naturally selected traits, Z scores and the proportion of the normal curve that lies beyond a given Z score. Sources: (1) Arnqvist 1992; (2) Badyaev & Hill 2000; (3) Badyaev & Martin 2000; (4) Badyaev et al. 2000; (5) Cohn 1990; (6) Fairbairn & Preziosi 1996; (7) Forslund 2000; (8) Loftus-Hills & Littlejohn 1992; (9) Pryke et al. 2001.

Taxonomic group	Trait	CV (%)	Z score	P	Source
Aquarius remigis (Heteroptera, Gerridae)	Total length	4.21	-0.16	0.436	6
Aquarius remigis (Heteroptera, Gerridae)	Prefemoral width	4.81	0.16	0.436	6
Aquarius remigis (Heteroptera, Gerridae)	Mesofemoral width	4.64	0.06	0.476	6
Carpodacus mexicanus (Aves, Fringillidae)	Culmen length	5.42	0.48	0.315	3
Carpodacus mexicanus (Aves, Fringillidae)	Wing length	2.25	-1.21	0.115	3
Carpodacus mexicanus (Aves, Fringillidae)	Body mass	7.09	1.37	0.085	3
Carpodacus mexicanus (Aves, Fringillidae)	Culmen length	3.52	-0.53	0.298	2
Carpodacus mexicanus (Aves, Fringillidae)	Wing length	2.46	-1.09	0.137	2
Carpodacus mexicanus (Aves, Fringillidae)	Tarsus length	3.15	-0.73	0.232	2
Carpodacus mexicanus (Aves, Fringillidae)	Body mass	6.45	1.03	0.151	2
Carpodacus mexicanus (Aves, Fringillidae)	Culmen length	4.02	-0.26	0.397	4
Carpodacus mexicanus (Aves, Fringillidae)	Wing length	2.44	-1.11	0.133	4

Carpodacus mexicanus (Aves, Fringillidae)	Tarsus length	3.50	-0.54	0.294	4
Carpodacus mexicanus (Aves, Fringillidae)	Body mass	5.89	0.73	0.232	4
Euplectes ardens (Aves, Ploceidae)	Collar area	8.35	2.04	0.020	9
Euplectes ardens (Aves, Ploceidae)	Culmen length	2.66	-0.99	0.161	9
Euplectes ardens (Aves, Ploceidae)	Tarsus length	1.31	-1.71	0.043	9
Euplectes ardens (Aves, Ploceidae)	Wing length	1.63	-1.54	0.061	9
Euplectes ardens (Aves, Ploceidae)	Body mass	5.36	0.45	0.326	9
Forficula auricularia (Dermaptera, Forficulidae)	Elytra width	5.87	0.72	0.235	7
Forficula auricularia (Dermaptera, Forficulidae)	Elytra length	8.73	2.25	0.012	7
Forficula auricularia (Dermaptera, Forficulidae)	Pronotum width	7.17	1.41	0.079	7
Gastrophryne olivacea (Anura, Microhylidae)	Body length	7.14	1.39	0.082	8
Gastroprhyne olivacea (Anura, Microhylidae)	Body length	5.41	0.48	0.315	8
Gerris odontogaster (Heteroptera, Gerridae)	Length of middle legs	2.95	-0.84	0.200	1
Gerris odontogaster (Heteroptera, Gerridae)	Length of middle legs	3.37	-0.61	0.270	1
Gerris odontogaster (Heteroptera, Gerridae)	Length of middle legs	3.56	-0.51	0.305	1
Gerris odontogaster (Heteroptera, Gerridae)	Length of anterior femur	3.22	-0.69	0.245	1

Gerris odontogaster (Heteroptera, Gerridae)	Length of anterior femur	3.27	-0.67	0.251	1
Gerris odontogaster (Heteroptera, Gerridae)	Length of anterior femur	4.37	-0.08	0.468	1
Gerris odontogaster (Heteroptera, Gerridae)	Elytra width	4.04	-0.26	0.397	1
Gerris odontogaster (Heteroptera, Gerridae)	Elytra width	3.06	-0.78	0.217	1
Gerris odontogaster (Heteroptera, Gerridae)	Elytra width	3.03	-0.79	0.214	1
Nephila clavipes (Aranae, Araneidae)	Palp length	5.33	0.43	0.333	5
Nephila clavipes (Aranae, Araneidae)	Conductor length	4.91	0.21	0.416	5
Nephila clavipes (Aranae, Araneidae)	Conductor length	4.90	0.20	0.420	5
Nephila clavipes (Aranae, Araneidae)	Conductor width	7.71	1.70	0.044	5

APPENDIX II

Coefficients of variation (CV) of sexually selected traits, Z scores and the proportion of the normal curve of naturally selected traits that lies beyond a given Z score. Sources: (1) Badyaev & Young 2004; (2) Fairbairn & Preziosi 1996; (3) Forslund 2000; (4) Klappert & Reinhold 2003; (5) Lüpold et al. 2004; (6) Markow et al. 1996; (7) Møller & Petrie 2002; (8) Pryke et al. 2001; (9) Regosin & Pruett-Jones 2001; (10) Reid et al. 2005; (11) Warner & Schultz 1992.

Taxonomic group	Trait	CV (%)	Z score	P	Source
Aquarius remigis (Heteroptera, Gerridae)	Wing shape	74.34	37.24	0.000	2
Carduelis flammea (Aves, Fringillidae)	Visible area of ornamentation	36.60	17.11	0.000	1
Carduelis flammea (Aves, Fringillidae)	Hue (ornamentation)	16.30	6.28	0.000	1
Carpodacus mexicanus (Aves, Fringillidae)	Visible area of ornamentation	29.00	13.06	0.000	1
Carpodacus mexicanus (Aves, Fringillidae)	Hue (ornamentation)	18.90	7.67	0.000	1
Chorthippus biguttulus (Orthoptera, Acrididae)	Attractiveness of two-leg males	44.33	21.23	0.000	4
Chorthippus biguttulus (Orthoptera, Acrididae)	Loudness of two-leg males	26.94	11.96	0.000	4
Chorthippus biguttulus (Orthoptera, Acrididae)	Pause/syllable ratio of two-leg males	19.23	7.85	0.000	4
Drosophila pseudoobscura (Diptera, Drosophilidae)	Bristle number of mating males	15.74	5.98	0.000	6
Drosophila pseudoobscura (Diptera, Drosophilidae)	Upper sex comb of mating males	23.67	10.21	0.000	6
Drosophila pseudoobscura (Diptera, Drosophilidae)	Lower sex comb of mating males	53.67	26.21	0.000	6

Drosophila simulans (Diptera, Drosophilidae)	Bristle number of mating males	15.92	6.08	0.000	6
Drosophila simulans (Diptera, Drosophilidae)	Sex comb number of mating males	9.75	2.79	0.002	6
Euplectes ardens (Aves, Ploceidae)	Tail length	16.85	6.58	0.000	8
Forficula auricularia (Dermaptera, Forficulidae)	Forceps length	19.92	8.21	0.000	3
Forficula auricularia (Dermaptera, Forficulidae)	Abdomen length	16.54	6.41	0.000	3
Forficula auricularia (Dermaptera, Forficulidae)	Body mass	26.20	11.57	0.000	3
Melospiza melodia (Aves, Emberizidae)	Song repertoire size	22.60	9.64	0.000	10
Nyctalus noctula (Chiroptera, Vespertilionidae)	Penis length	11.50	3.72	0.000	5
Passer domesticus (Aves, Passeridae)	Visible area of ornamentation	26.60	11.78	0.000	1
Passer domesticus (Aves, Passeridae)	Hue (ornamentation)	13.80	4.95	0.000	1
Pavo cristatus (Aves, Phasianidae)	Train length	23.90	10.34	0.000	7
Pavo cristatus (Aves, Phasianidae)	No. of ocelli	84.00	42.39	0.000	7
Pavo cristatus (Aves, Phasianidae)	Diameter of ocelli	27.50	12.26	0.000	7
Thalassoma bifasciatum (Perciformes, Labridae)	Black + white area	26.55	11.75	0.000	11
Thalassoma bifasciatum (Perciformes, Labridae)	White area	32.87	15.12	0.000	11
Thalassoma bifasciatum (Perciformes, Labridae)	Tail length	23.33	10.03	0.000	11

Tyrannus forficatus (Aves, Tyrannidae)	Tail length (male)	13.60	4.84	0.000	9
Tyrannus forficatus (Aves, Tyrannidae)	Tail length (female)	14.00	5.06	0.000	9